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BY
CARSTEN SIMONSEN

DISSERTATION SUBMITTED 2018

AALBORG UNIVERSITY
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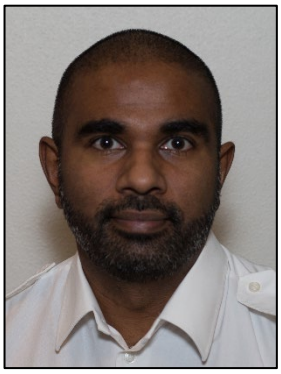

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ENGLISH SUMMARY

Carbon monoxide (CO) is an extremely poisonous gas, without smell, taste or colour. Therefore, it is hard to detect without the use of electronic CO alarms. Normally, only insignificant concentrations are present in the air we breathe, however, in connection with incomplete combustion of organic material, the concentration can rise markedly. Thus, smoke from fire and exhaust gas from cars (although less after the introduction of the catalytic converter), cause CO poisoning. The toxicity of CO is primarily based on the high affinity to ferric and ferrous iron which is found in several essential molecules in the human body i.e. hemoglobin, myoglobin and cytochrome c oxidase (complex IV). Of other toxic effects that should be mentioned are induction of inflammation, and increase in the formation of reactive oxygen species, which may cause damage to the cellular proteins i.e. DNA. These toxic reactions result in acute symptoms i.e. headache, dizziness, dyspnea, unconsciousness, heart failure and ultimately death. The treatment of CO poisoning has not changed much during the last 50-60 years and is entirely based on inhalation of 100% oxygen, either at normobaric conditions (NBO) or hyperbaric conditions (HBO) which in Denmark is provided by Rigshospitalet in Copenhagen. Extracorporeal membrane oxygenation (ECMO) is primarily used in the treatment of patients suffering from cardiac or pulmonary failure. By oxygenation of the blood outside the body and/or supporting cardiac function, it may be possible to buy time to execute intervention or to wait for the affected organ to restitute and regain its function.

The overall aim of this PhD project was to describe the extend of CO poisoning in Denmark. Additionally, to increase our knowledge regarding the acute management with focus on 1) the possibilities to use ECMO in the treatment of severely CO intoxicated patients and 2) use this technique during airborne transportation of patients over long distances using fixed wing aircrafts.

To gain insight into the above mentioned issues, an epidemiological study and two experimental studies using animal models, were performed.

In study 1, the incidence of CO poisoning in Denmark, during the period from 1995-2015, was evaluated, with focus on, how potential co-morbidities are associated to mortality. Additionally, evaluation of how HBO treatment was associated with survival was done. This study was a retrospective follow-up study using several databases at Statistics Denmark. In study 2 and study 3, experimental animal models (pigs) were utilized. In Study 2, 12 pigs were used. They were poisoned with CO until cardiac failure, and then they were randomized to either ventilator treatment using FiO_2 at 100% or ECMO treatment. Simultaneously the pulmonary vascular resistance (PVR) was measured and lung biopsies were secured in order to reveal any potential damage to the lung tissue. Study 3 was a feasibility study, in which a pig was

poisoned with CO and then subjected to a flight at 8,000 feet, using a Hercules C130J from the Danish Airforce. During flight, cardiac arrest was induced in the pig followed by testing of the feasibility of performing in air cannulation.

In Study 1, data from 22,930 persons was analysed. Approximately 1,000 persons are poisoned with CO each year and approximately 100 persons die from CO poisoning annually. The overall mortality was 9.2%, of which the majority did not reach a hospital alive (85%). Among the patients who survived the initial 30 days after poisoning, no difference in survival was found when comparing those who received HBO treatment to those who did not, once various co-morbidities were taken into account. The comorbidities that were associated with increased mortality, were in general those typically associated to impaired cognitive or physical condition. Among those who died from exposure to gas and not fire smoke, 98% were intentional poisonings.

In study 2, a significantly increased survival among the research animals in the group treated with ECMO was found. All six pigs survived beyond complete discontinuation of ECMO. Contrary, only one pig in the ventilator group survived, while the five others went into cardiac arrest that could not be reversed using conventional advances resuscitation methods. However, after these resuscitation efforts were abandoned, four out of these five pigs were then resuscitated using ECMO treatment after which they could be weaned completely. Even though a very high arterial oxygen pressure (PaO_2) was reached, when using ECMO treatment, this did not reflect as a decrease in the half-life of HbCO. There was no correlation between CO poisoning and the PVR and also no histological changes in the lung tissue samples.

In study 3, it was demonstrated that performing a flight with a CO poisoned pig in a Hercules C130J aircraft is possible. Additionally it was demonstrated, apparently as a world first, that it is possible to perform cannulation and initiate ECMO treatment during flight.

Further research is required to explore, how ECMO treatment can be implemented in the acute treatment of CO poisoning. By using ECMO treatment during flight, or as a possible rescue therapy, we might be able to perform fixed wing airborne transportation of patients that otherwise would have been too critically ill for aerial transportation.

DANSK RESUME

Kulilte er en yderst giftig gasart, som hverken har nogen lugt, smag eller farve. Det er derfor svært at opdage den uden brug af elektroniske alarmer. Normalt er der kun ubetydelige mængder af kulilte i den luft vi indånder, men i forbindelse med ufuldstændig forbrænding af organiske materialer kan koncentrationen stige markant. Således vil bl.a. røg fra ild og udstødningsgas fra biler (dog i mindre grad efter indførelse af katalysatoren), kunne medføre forgiftning. Kuliltes toksicitet skyldes primært den store affinitet til jern-ioner som findes i flere essentielle molekyler af betydning for homoeostasen, såsom hæmoglobin, myoglobin og cytocrom c oxidase (kompleks IV). Af andre effekter kan nævnes induktion af inflammation, samt dannelse af reaktive oxygen species (ROS), der medfører dannelse af frie radikaler, som potentielt kan forårsage skade på arvemassen (DNA). De toksiske skadevirkninger medfører i det akutte forløb symptomer som hovedpine, svimmelhed, åndedrætsbesvær, bevidstløshed, hjertesvigt og kan i yderste konsekvens medføre døden. Behandlingen af kulilteforgiftning har ikke ændret sig nævneværdigt gennem de seneste 50-60 år og består af inhalation af 100% ilt, enten ved normalt tryk (NBO) eller ved højt tryk i et trykkammer (HBO) som findes på Rigshospitalet. Ekstrakorporal cirkulation (ECMO) bliver almindeligvis anvendt til behandling af patienter med hjerte- eller lungesvigt. Ved at ilte blodet uden for kroppen/overtage hjertets funktion kan man købe sig tid til at foretage intervention, eller til at det påvirkede organ kan komme sig og varetage sin funktion igen.

De overordnede mål med dette ph.d. projekt var at beskrive omfanget af kulilteforgiftning i Danmark. Desuden at øge vores viden omkring aspekter ved den akutte behandling med hovedvægt på at undersøge 1) mulighederne for brug af ECMO som behandling af svært kulilteforgiftede patienter og 2) anvendelse af denne teknik ved luftbåren transport af patienterne over længere distancer med fly.

For at belyse ovenstående problematikker udførte vi et registerbaseret epidemiologisk studie samt to dyreeksperimentelle studier.

I studie 1, kiggede vi på forekomsten af kulilteforgiftning i Danmark i perioden 1995-2015 med særligt fokus på, hvorledes eventuelle co-morbiditeter indvirkede på mortaliteten. Desuden kiggede vi også på, hvorledes HBO-behandling var associeret med overlevelsen. Der er tale om et retrospektivt follow-up studie med anvendelse af forskellige databaser hos Danmarks Statistik. I studie 2 samt studie 3 anvendte vi en dyremodel med grise. I studie 2 indgik 12 grise som blev forgiftet med kulilte indtil de fik hjertesvigt, hvorefter de blev randomiseret til enten respiratorbehandling med 100% ilt eller ECMO-behandling. Samtidig målte vi modstanden i lungekredsløbet, samt tog lungebiopsier for at afsløre eventuelle histologiske alveoleskader. Studie 3 var et feasibility studie, hvor vi kulilteforgiftede en gris og derefter foretog en

flyvning i 8.000 fod i et Hercules C130J fly fra Forsvaret. Undervejs inducerede vi et hjertestop på grisen, hvorefter vi testede om kanylering under flyvning var praktisk muligt.

I studie 1 analyserede vi data fra 22.930 personer. Vi fandt, at ca. 1000 personer bliver forgiftet med kulilte årligt i Danmark, og at ca. 100 personer dør om året som følge af kulilteforgiftning. Den samlede mortalitet var 9,2%, hvoraf størsteparten blev erklæret døde uden at komme på hospitalet (85%). Blandt dem, der overlevede de første 30 dage efter forgiftningen, var der ikke nogen forskel på overlevelsen blandt dem, der modtog HBO-behandling vs. dem, som ikke fik HBO behandling, når man korrigerede for diverse co-morbiditeter. De co-morbiditeter der vat associeret med øget dødelighed var dem som man typisk forbinder med dårlig kognitiv eller fysisk funktion. Blandt dem, som omkom, hvor eksponeringskilden var gas og ikke røg, var 98 % selvmord.

I studie 2 var der markant øget overlevelse blandt de forsøgsdyr, der blev behandlet med ECMO. Alle 6 grise overlevede og kunne til sidst klare sig uden ECMO-støtte. Kun én gris i respiratorgruppen overlevede, mens resten fik hjertestop og ikke kunne genoplives med konventionel avanceret hjertelungeredning. Efter genoplivningsforsøg blev opgivet, lykkedes det dog, ved hjælp af ECMO, at få 4 ud af disse 5 grise til at overleve til et punkt, hvor også de kunne klare sig uden ECMO-støtte. Selvom vi kunne opnå et meget højt ilttryk i blodet hos grisene ved anvendelse af ECMO, havde det ikke nogen gavnlig effekt på halveringstiden af HbCO. Vi fandt ikke en sammenhæng mellem forgiftning og modstanden i lungekredsløbet, og heller ikke nogen histologiske forandringer i lungevævet.

I studie 3 demonstrerede vi, at det kan lade sig gøre at foretage en flyvning med en kulilteforgiftet gris i et Hercules C130J fly. Tillige demonstrerede vi, tilsyneladende som de første i verden, at det er muligt at foretage kanylering og igangsætte ECMO behandling under flyvning.

Yderligere forskning er påkrævet for at undersøge, hvorledes ECMO-behandling kan implementeres i den akutte behandling af kulilteforgiftning. Ved at anvende ECMO-behandling under flyvning, eller eventuelt have muligheden for at kanylere og starte ECMO-behandling undervejs, kan man potentielt flytte patienter, som ellers ville være for kritiske til transport med fly.

ACKNOWLEDGEMENTS

The present PhD thesis represents work carried out while employed as a clinical assistant from 2016 to 2018 at the Department of Cardiothoracic Surgery, Aalborg University Hospital.

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First of all, I would like to thank my main supervisor, Professor Jan Jesper Andreasen, I am forever grateful for the opportunity to pursue this PhD. Jan has been extremely helpful, in all aspects of the completion of this project, and has offered both guidance and freedom in just the right amount.

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I am grateful for the cooperation with my friends and colleagues at the AIREVAC Squadron 690, at Aalborg airbase. Especially the support provided by Dr René Bleeg and Dr Claus Lie, was essential for the completion of the airborne parts of the experiments, but also the supporting staff at SQN 690, showed great enthusiasm towards the project. I look forward to continue this great collaboration in future projects. I thank The Royal Danish Armed Forces Health Services for approving the collaboration and the project in general, and for support in planning as well as execution of the flights.

A also want to express my gratitude to Professor Christian Torp-Pedersen, for invaluable help with initiating and shaping of the epidemiological part of this project, to my friend and colleague, Kristinn Thorsteinson, for all the help with statistical coding and data crunching. I am grateful for the help provided by statistician Rikke Nørmark, who, on several occasions patiently answered and explained.

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University Hospital, as these contributions ensured the completion of this project within the provided time frame.

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LIST OF ABBREVIATIONS

ARDS	Adult respiratory distress syndrome
CABG	Coronary artery bypass grafting
CO	Carbon monoxide
COPD	Chronic obstructive pulmonary disease
CPS	Cardio pulmonary assist
DNA	Deoxyribonucleic acid
ECMO	Extracorporeal membrane oxygenation
ECPR	Extracorporeal pulmonary resuscitation
FiO_2	Fraction of inspired oxygen
ft	Feet
G-force	Gravitational force
H&E	Haematoxylin and eosin
Hb	Haemoglobin
HbCO	haemoglobin-CO
HBO	Hyperbaric oxygen
ICD	International classification of diseases
iLA	Interventional lung assist
MEDEVAC	Medical evacuation
NBO	Normobaric oxygen
PaO_2	Arterial oxygen pressure
PECLA	Pulmonary extracorporeal lung assist
PPMV	Parts per million volume
PVR	Pulmonary vascular resistance
RDAF	Royal Danish Air Force
ROS	Reactive oxygen species
VA	Veno-arterial
VV	Veno-venous

CONTENT

TABLE OF FIGURES

Unless otherwise specified, the figures included in this PhD thesis are created by the author or in the public domain.

Figure 5.9. Cannulation was performed and ECMO treatment was established.

Table 5.1. Major events, altitude, vital values and arterial blood gas results obtained during the experiment. (Study 3)

Figure 6.1. Intensive care MEDEVAC module - exterior and interior (photos courtesy of Jean-Michel Ferrieux).

Figure 6.2. The left image shows the interior of a C-17 aircraft during an aeromedical evacuation of patients from Iraq to Germany. The right image shows a patient on veno-venous extracorporeal circulation transported in a C-17 aircraft from Japan to Hawaii.

1 SCIENTIFIC PAPERS

This PhD thesis is based on three independent scientific papers. These papers are referred to by use of Arabic numbers (1-3) in the order listed below.

1.
Simonsen C, Thorsteinsson K, Mortensen RN, Torp-Pedersen C, Kjærgaard B, Andreasen JJ. Carbon monoxide poisoning in Denmark with focus on mortality and factors contributing to mortality. Submitted to PLOS One.

2.
Simonsen C, Magnusdottir SO, Andreasen JJ, Rohde MC, Kjærgaard B. ECMO improves survival following cardiogenic shock due to carbon monoxide poisoning - an experimental porcine model. Scand J Trauma Resusc Emerg Med 2018;26:103. doi:10.1186/s13049-018-0570-6.

3.
Simonsen C, Magnusdottir SO, Andreasen JJ, Bleeg RC, Lie C, Kjærgaard B. Long distance transportation of CO-poisoned patients on ECMO seems possible – a porcine feasibility study. Submitted to Air Medical Journal.

2 BACKGROUND

2.1 CLINICAL ASPECTS OF CARBON MONOXIDE POISONING

BIOCHEMISTRY

Carbon monoxide (CO) consists of one carbon atom and one oxygen atom bound together via three covalent bonds (Figure 1.1). CO freezes at -205 °C and evaporates at -191.5 °C (1 atm) and therefore appears in the form of a gas under normal temperature and pressure conditions (3). CO has approximately the same density as the surrounding atmospheric air, and CO concentrations at sea level are approximately 0.04-0.12 parts per million volume (ppmv), depending on geography (highest in the northern hemisphere). Peak concentrations of approximately 0.2 ppmv have been observed in winter (4). Higher concentrations of CO typically occur in proximity to sites with incomplete combustion of organic materials, particularly in the presence of a low oxygen concentration:

$C_xH_y + zO_2 \rightarrow aCO_2 + bCO + cH_2O + dH_2$

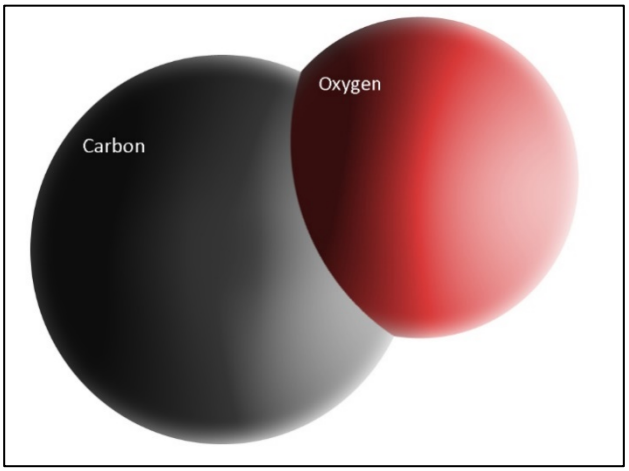

Figure 2.1. Carbon monoxide molecule.

CO binds strongly to both ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron. This binding is the leading cause of its toxic properties, as these iron ions have essential functions in vital molecules in the human body, as described in the following sections.

HISTORY AND SOURCES OF CARBON MONOXIDE

As long as humans have harnessed fire, the risk of CO poisoning has been imminent. The first written evidence of CO poisoning was recorded by Aristotle in the third century BC in a description of how animals react to fumes. He writes "*They are destroyed, however, by these things, just as human beings are; i.e., as human beings get headaches from, and are often asphyxiated by, the fumes of charcoal, so the lower animals perish from the strong fumes of brimstone and bituminous substances*" (5). The great Carthaginian conqueror Hannibal is said to have used CO to execute prisoners during the First Punic War in 264-241 BC by lighting coal inside enclosed bathing rooms (6). Indoor coal burning poisoned the Byzantine emperor Julian in approximately 350 AD and probably killed his successor Jovian in 364 (7). However, it was not until 1842 that the French chemist and surgeon Nicolas Leblanc identified CO as the poisonous component of coal gas (6). At approximately the turn of the twentieth century, the internal combustion engine became commercially available (8) and thus gave rise to a new source of CO poisoning. To this day, CO produced by internal combustion engines remains a significant source of poisoning, as these engines are found in a wide range of vehicles (9–11), sea vessels (12,13) and power tools/electric generators (14). Notably, diesel-fuelled engines pose a very low risk compared to gasoline-fuelled engines (15). Another important source of CO poisoning is fire, and several forensic studies suggest that CO is the major cause of mortality in fire-related deaths and that heat/burns are of minor importance (16,17). The last of the important sources of CO poisoning is residential heat appliances, either as a result of leakage of the gas supply or defective emission of the waste fumes created by the combustion of the gas/fuel used to generate the heat (18,19). The use of residential heat appliances parallels the increased number of cases of CO poisoning in winter (20,21). Smoking can increase the level of Hemoglobin-CO (COHb) in the blood (22). This increase is normally moderate, as indicated by the results of a non-invasive CO-oximetry screen in an emergency department in the US reported in 2008 by Suner et al. (23). Notably, 10,856 patients were screened and presented average COHb levels of 5.17% (± 3.78) among smokers and 2.9% (± 2.76) among non-smokers. However, in some cases, much higher COHb levels occur as a result of smoking (24) and causal reports of COHb levels of 35% exist (25).

The rare sources of CO poisoning mentioned in the literature include defective liquid petroleum gas (LPG)-fuelled water heaters (26,27), water pipe smoking (28–30), and the mixing of formic acid with strong acids (31,32). Unfortunately, CO has also been used during warfare and was used in the genocide of Jews during World War II (33).

As described above, the toxicity of CO has been well known for centuries. In the next section, I will describe the more specific biological mechanisms of poisoning.

TOXIC EFFECTS OF CO

As mentioned above, CO binds tightly to ferrous and ferric iron, which is an important component of several essential molecules in the human body, leading to a range of toxic effects. One of these molecules is haemoglobin (Hb) (Figure 2.4a). The Hb molecule contains four haem groups, each with a ferrous iron atom, which, under normal conditions, is used to transport O_2 from the lungs to the various tissues in the body and CO_2 away from the tissue and back to the lungs. The crude affinity of CO for ferrous iron is approximately 100,000 times stronger than O_2; however, when ferrous iron is integrated in the Hb molecule this ratio is reduced by a factor of approximately 1,000. This strong affinity was first suspected by C. Bernard in 1857 (34) and confirmed by J.S. Haldane (Figure 2.2a) in 1895 (35) in a study where he also noticed that the introduction of oxygen to organisms subjected to CO poisoning might improve the condition. Later, the binding of Hb and CO was described in more detail by C. Douglas and J.S. Haldane and his son J.B.S Haldane (Figure 2.2b) in 1927 (36), when they constructed a Hb-CO dissociation curve illustrating how the amount of inspired oxygen is related to the saturation of Hb with CO (Figure 2.3a).

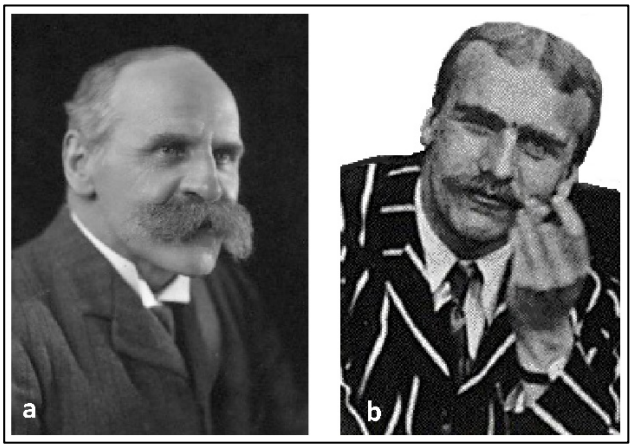

Figure 2.2. a) J.S. Haldane (1910) and b) his son J.B.S. Haldane (1914).

In the equilibrium that describes of the formation of HbO_2 and HbCO ($HbO_2 + CO \rightleftharpoons HbCO + O_2$), only small concentrations of CO increase the formation of HbCO, whereas increase in the percentage of inspired oxygen facilitates the desaturation of CO from Hb (Figure 2.3b). The authors also discovered a leftward shift in the oxygen-Hb dissociation curve in the presence of CO. This shift (the Haldane shift) impairs oxygen dissociation from Hb in tissues when Hb is partially saturated with CO.

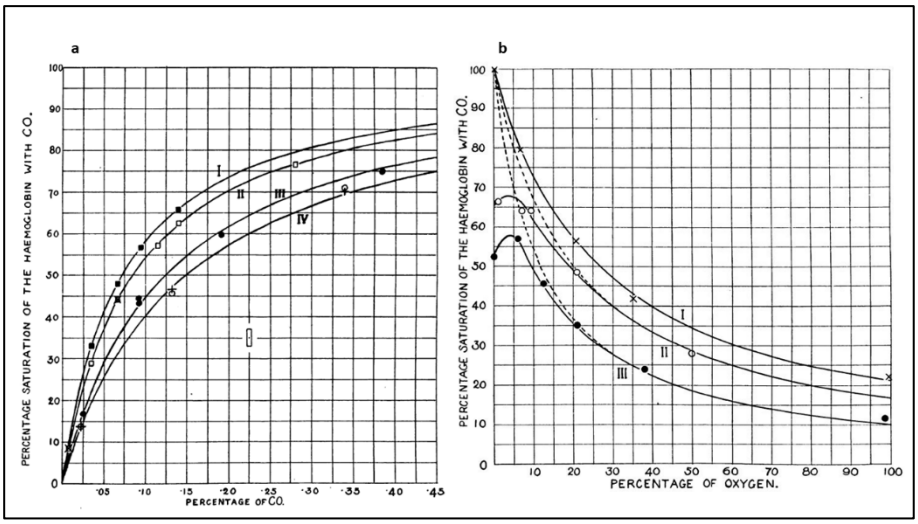

Figure 2.3. a) The relation between the binding of CO to Hb depends on the concentration of inspired CO in two humans (upper curves) and two mice (lower curves). b) The relationship between the saturation of Hb with CO depends on the percentage of inspired oxygen when the CO concentration is constant (below 0.1%) in a human (upper curve) and mice (lower two curves) Doulgas C. & Haldane J.S., 1912.

As only 2% of the oxygen content in the blood is dissolved in the plasma (37) at a normal temperature/pressure, the expanded oxygen-carrying capacity of Hb is essential to ensure normoxic conditions in the tissues. When the oxygen-carrying capacity of Hb is reduced by competitive CO binding, sufficient tissue oxygenation is compromised. The binding of CO to Hb is approximately 210 times stronger than oxygen (38).

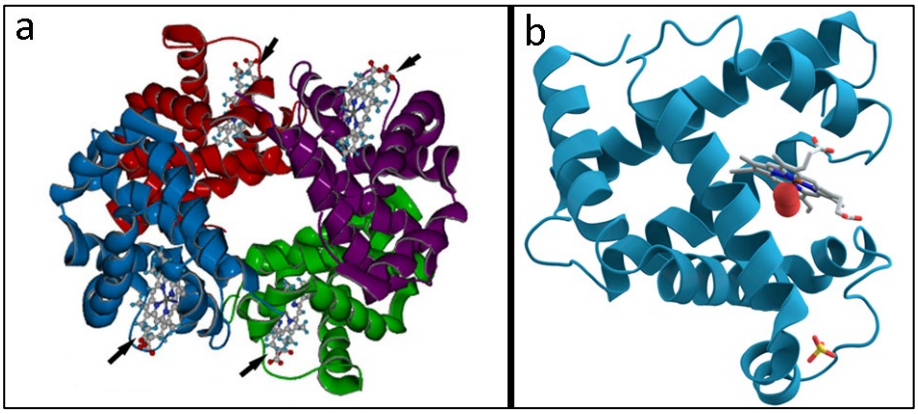

Figure 2.4. a) Structure of the haemoglobin molecule, including the four haem-groups – marked by arrows (created by Bielabio). b) Structure of the myoglobin molecule.

J.B.S. Haldane noticed that the poisonous effects of CO seemed to extend beyond the effects on Hb. In a paper from 1927 (39), he stated "*The movements of a moth and the germination of cress seed are inhibited by CO. The greater the partial pressure of 0_2 the more CO is required.*" Neither moth nor cress use haemoglobin for oxygen transport. Therefore, he wrote in the paper: "*It is concluded that cells contain a catalyst of oxidation which is poisoned by CO.*" Several decades later, this catalyst was identified as cytochrome c oxidase (Figure 2.5) (40,41).

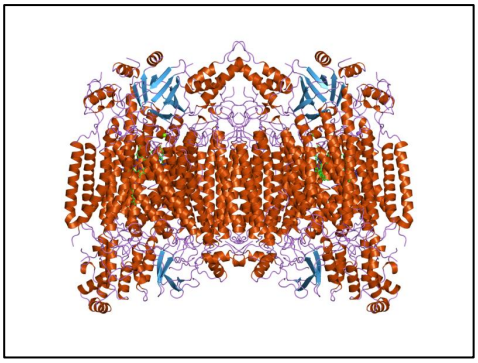

Figure 2.5. Structure of the mitochondrial cytochrome c oxidase (Complex IV) – (courtesy of The European Bioinformatics Institute)

Cytochrome c oxidase (complex IV) reduces oxygen, which forms water and pumps protons from the mitochondrial matrix into the intracellular space (Figure 2.6). Protons are used to produce ATP and are therefore essential in cellular energy metabolism, which is compromised by CO poisoning. Another well-known toxic agent that acts by inhibiting cytochrome c oxidase is cyanide (42).

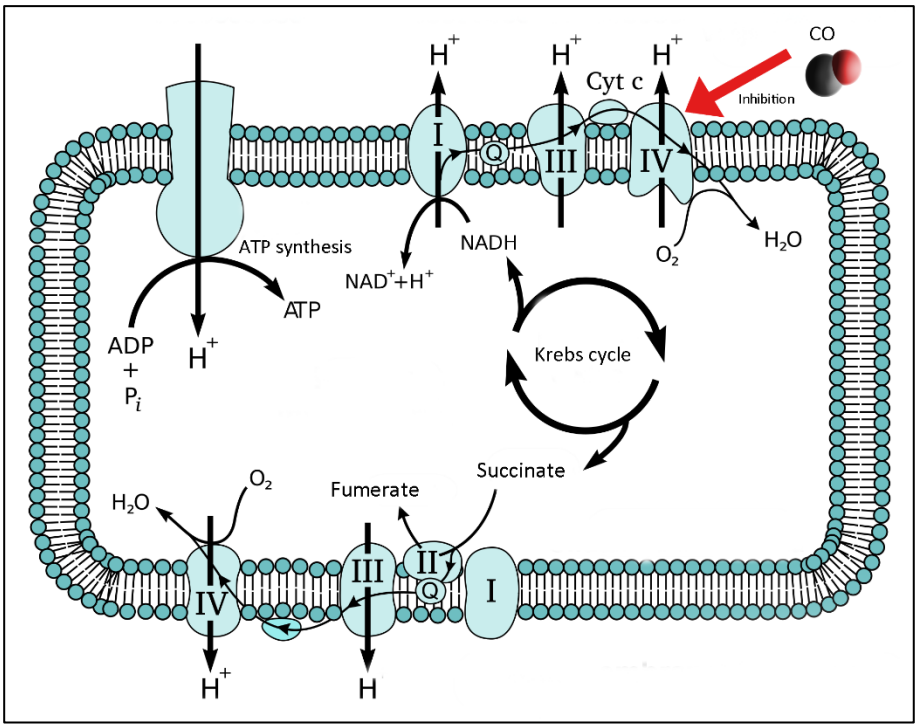

Figure 2.6. Mitochondria. CO inhibits cytochrome c oxidase (complex IV) in the electron transport chain.

The inhibitory effect of CO on cytochrome c oxidase extends beyond the normalization of the percentage of HbCO in blood and it is therefore suspected to play a role in the development of the long-term effects of CO poisoning (40).

Myoglobin also contains a haem group with ferrous iron (Figure 2.4b) and transports oxygen in red muscle cells/heart muscle cells under normal conditions, where it carries oxygen to the mitochondria. Myoglobin also functions as an oxygen reservoir that may serve as a buffer under ischemic conditions. CO binds to the haem group with greater affinity than oxygen and thereby inhibits oxygen storage/transport, potentially leading to diminished contractility of heart muscle cells (43–45).

CO intoxication induces an immune response by activating neutrophils (46) (47) and subsequently inducing the formation of reactive oxygen species (ROS), which facilitates the development of free radicals (48). CO poisoning also causes changes in the both neuronal and cardiac potassium ion channels, resulting in increased excitability (49). These changes, along with hypoxic damage and the inflammatory and immunological responses, may be the foundation for the neurological sequelae that are frequently associated with CO poisoning (50–53).

Regarding the impact of CO on pulmonary function, several papers have examined the effects of low concentrations of CO on pulmonary vascular resistance (PVR). Low concentrations of CO in inspired air reduce the pulmonary pressure by decreasing pulmonary vascular resistance (54–56). In cases of severe CO poisoning, damage to the epithelial layer of the alveoli have been described. In a paper by Fein et al., the authors discovered increased alveolar epithelial permeability following CO intoxication in a rabbit model that potentially caused pulmonary oedema and thereby decreased gas exchange (57). This finding is supported by a case report from 1980 (58). However, compared to the number of cases of CO poisoning, pulmonary oedema is not frequently described. Further information about the effects of severe CO poisoning on the pulmonary circulation, PVR and the alveoli is needed to obtain a better understanding of the harmful effects of CO.

CO poisoning results in a prominent risk of developing acute heart failure (59–61), and cardiac arrest which has a very high mortality rate. This was demonstrated in a study performed in 2001 that included 18 patients suffering from cardiac arrest (62). Despite extensive efforts, including HBO treatment, all patents died. Additionally, long-term cardiac effects have been reported, such as arrhythmias (63) and major adverse cardiovascular events (64).

Symptoms associated with acute CO intoxication include headache, vertigo, nausea, visual impairment, palpitations, loss of consciousness, and coma, and can ultimately lead to death (65). In addition to the risk of imminent death, CO poisoned patients experience an increased risk of developing neurological symptoms i.e., dementia (66), extrapyramidal symptoms (53), and encephalopathy (67). Long-term survival is also decreased following CO poisoning (68).

CURRENT TREATMENT FOR CO INTOXICATION

Current treatment is generally based on increasing the oxygen supply for two main reasons: 1) to increase oxygen delivery to the tissues and 2) to increase the dissociation of CO from Hb and thereby increase the elimination of CO (69).

J.S. Haldane was a pioneer in the treatment of CO poisoning. In a paper from 1895, he reported the possible benefits of using oxygen to treat CO poisoning in an animal experiment using mice (70). He also introduced the idea of using hyperbaric oxygen when he showed that rats survived CO poisoning when they were placed in a chamber containing oxygen at a pressure of 2 atmospheres. Furthermore, he also developed equipment for NBO (Figure 2.7) (1). The first application of the HBO to treat a patient with CO poisoning occurred in 1960 (71).

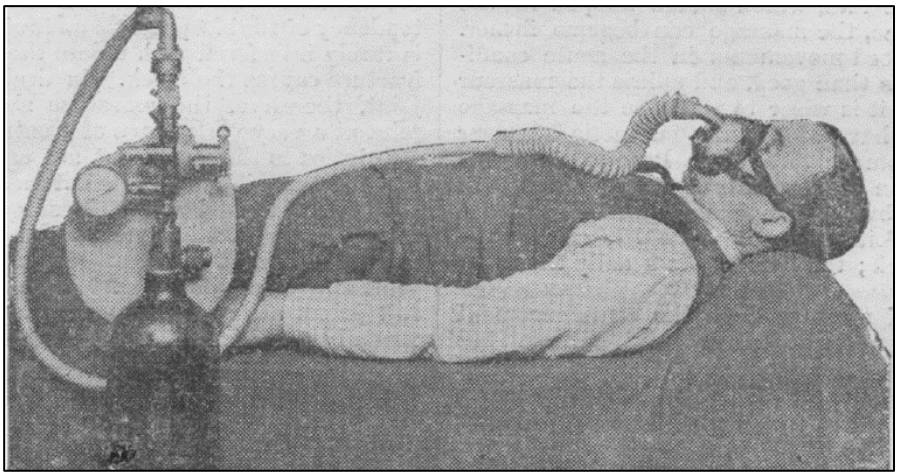

Figure 2.7. Normobaric oxygen therapy in 1917, J.S. Haldane (1)

Under normobaric conditions, the administration of oxygen reduces the HbCO half-life from approximately 320 minutes to 74 minutes (69), and an HBO treatment further reduces this value to approximately 20 minutes (47). Nevertheless, the effects of this reduction on mortality/morbidity are not completely evident. In a Cochrane review from 2011, the authors performed a meta-analysis of six previously published randomized controlled trials examining neurological sequelae and concluded that "*Existing randomized trials do not establish whether the administration of HBO to patients with carbon monoxide poisoning reduces the incidence of adverse neurologic outcomes*" (72). Although evidence supporting the opposite conclusion has been reported (73), a recent study from 2018 involving 25,737 patients in Taiwan actually identified an increased risk of neurological sequelae, even after considering co-morbidities (74). Only one published retrospective study claims that HBO treatment is associated with decreased mortality (acute and 1-year) (75). Additional randomized controlled trials are needed to clarify the effects of HBO treatment on both the mortality and morbidity of patients with CO poisoning.

No broadly accepted guidelines are available for the indications to offer HBO treatment (76). This limitation, along with the use of different protocols for treatment and the delay in the initiation of HBO treatment due to logistic challenges, may explain some of the inconsistencies in the reported benefits of HBO treatment. In Denmark, the criteria for offering HBO are (77): neurological symptoms that are more severe than an ordinary headache, a patient who is or has been unconscious, the presence of cardiac arrhythmias or decreased cardiac function, an HbCO level greater than 25% measured at any point, and pregnant patients. Although a hyperbaric chamber exists in Aarhus, HBO therapy is primarily performed at Rigshospitalet in Copenhagen.

Hyperbaric oxygen therapy has been reported to be associated with adverse events, such as pulmonary oedema, barotrauma and seizures (78,79), and requires the patient to be cooperative. Additionally, the access to HBO therapy for the individual patient may be limited by geographical restrictions, as HBO typically is only offered at selected medical facilities within each country.

Several other strategies aiming to increase CO elimination have been proposed. Corbogen, a gas mixture of oxygen and 5-10% CO_2 may have the potential to decrease the COHb half-life to a similar extent as HBO (80). Carbon dioxide does not bind to Hb in the same way as oxygen, as it is bound to the protein chains of the Hb molecule rather than the ferrous iron of the haem group. As a result, carbon dioxide does not compete with oxygen in this binding process. Based on the capabilities of CO_2 to increase the elimination of CO, exercise has been shown to significantly decrease the CO half-life in patients with moderate CO poisoning (81). In an animal study, $ECMO_3$ (ECMO using ozone) treatment has also been shown to decrease the half-life of CO (82). Another animal study reported the doubling of the CO elimination rate following exposure to light with a wavelength of 532-628 nm (83). Finally, the use of antioxidants to minimize the effects of ROS has also been suggested as part of the treatment for CO poisoning (50).

Altogether, the treatment for CO poisoning has not changed substantially during the last 50-60 years. With our new and improved understanding of the toxic mechanisms of CO, we now have increased opportunities to search for more effective treatment protocols both in the acute phase of poisoning and strategies that will diminish the subsequent neurological impacts. The administration of ECMO in the initial phase of treatment may be a piece of this puzzle.

2.2 EPIDEMIOLOGY OF CO POISONING

EPIDEMIOLOGY WORLDWIDE

CO poisoning is regarded as the most common form of poisoning (84,85). In the US, approximately 50,000 cases of CO poisoning result in visits to emergency departments each year (86). This value equals an incidence of approximately 16 cases per 100,000 people/year. The annual number of deaths was estimated to be 2,700 in a paper from 2008. More recent studies suggest a decreasing trend, with 1,245 deaths reported in 2014 (87). The decline was due to a significant reduction in the number of deaths caused by intentional CO poisoning.

In 2013, the World Health Organization (WHO) Regional Office for Europe published a paper regarding deaths caused by CO poisoning in 28 member states (88). They collected data regarding 140,490 deaths from 1980-2008. However, the reporting was very inconsistent between countries, as differences in coding and information were evident across registries and the death rate varied from 0.02 to 12.8 between countries. Six countries provided data regarding admissions caused by intoxication with CO. The total number was 31,473 in the study period, corresponding to an average rate of admissions of 2.33 per 100,000 people/year.

In Asia, several studies regarding the epidemiology of CO poisoning have also been performed. In a study from Wuhan, China, 156 deaths caused by CO poisoning were reported from 2009-2014, corresponding to 0.5 per 100,000 population/year (20). Although the manner of death was analysed, information about admissions was not included in this study. Indoor charcoal burning resulting in CO poisoning has been an increasingly popular method of committing suicide in Taiwan and Hong Kong in recent years (89). In a paper from Taiwan released in 2015, the authors also examined factors (symptoms) associated with mortality (90). Shock exhibited the strongest association with increased mortality. Other significant factors included hypothermia, hepatitis, renal failure and coma. The overall mortality rate was 7.3% and HBO therapy was administered to 18.8% of patients. Another paper from Taiwan also assessed the demographics of CO poisoned patients and noticed a higher proportion of mental illnesses among female patients (91). In this study, 24.2% of the CO intoxicated patients were treated with HBO.

The aforementioned studies used very different methods, yielding differences in the results. While these differences may simply reflect the differences in geography, health care systems, logistics and treatment protocols, more comprehensive studies will contribute to the total knowledge in this field. In particular, additional studies analysing the impacts of co-morbidities may help provide strategies to improve impact/diminish the effects of CO poisoning.

EPIDEMIOLOGY IN DENMARK

No comprehensive published studies regarding the epidemiology of CO poisoning in Denmark along with an exploration of co-morbidities exist. A national report from the Danish Health Institute published in 1995 established national guidelines for the treatment of CO poisoning (92). The authors used data from Statistics Denmark to estimate the total number of cases of CO poisoning in Denmark. One hundred ninety-two patients were identified from the Cause of Death Registry, and 151 patients from the Danish National Patient Registry (DNPR) were admitted following CO exposure, of which 12 died (included in the 192 patients). The total number of patients was 331 in 1992 and the mortality was 58%. The authors recommended HBO therapy for

severe cases of CO poisoning. In 2003, this report was reviewed and updated by the Danish Health Authority, but the recommendations were not changed (93). In 2000, 20 patients were treated with HBO. In 2001, this number increased to 33.

Other studies have a more specific forensic perspective. For example, a paper published by Nielsen et al. in 2014 focused on suicide by charcoal burning (94) as did Hansen et al. (95) in 2006. All deaths by poisoning in East Denmark from 1998-2002 were examined in another Danish forensic study by Johansen et al. published in 2006 (96). Of the non-narcotic-related deaths, 13% were caused by CO and/or cyanide n=57. This study also examined the manner of death (36 due to an accident, 12 due to suicide and 9 due to uncertain causes).

A previous specific and significant source of CO poisoning in Denmark was Town Gas. Town Gas is a centrally produced gas distributed by gas lines to individual homes and is a method for providing fuel for heat/cooking in larger Danish cities. The CO content has decreased over time and was finally phased out completely in 2007. The number of homes using Town Gas has also decreased. Previously, the CO content in town gas caused numerous deaths each year. From 1950-1959, 407 deaths were attributed to CO from town gas (299 suicides, 90 accidents, and 18 homicides) (97). Another survey published in 2007 by Thomasen and Gregersen(18) examined CO-related deaths from 1995-1999 and identified 22 cases in which town gas caused death of a total of 449 non-fire-related CO poisoning cases.

Altogether, none of the above studies provides an overview of the epidemiology of CO poisoning in Denmark and how it develops over time. No Danish studies consider co-morbidities in the evaluation of the mortality of CO poisoning. No studies investigating how psychiatric diseases are linked to CO poisoning have been published and only a few international studies investigating this connection exist (73,91,98). Thus, additional research studies investigating the impacts of various co-morbidities, including psychiatric diseases, on the risk of CO poisoning and the subsequent mortality are required. Along with analyses of risk factors, the manner of death and the impact of treatment with HBO therapy, these studies will provide information that may be helpful in the management of CO intoxicated patients.

2.3 EXTRACORPOREAL TREATMENT OF CO POISONING

ECMO BACKGROUND

Extracorporeal Membrane Oxygenation (ECMO) describes the principle of creating an external circulation in which blood is oxygenized through a synthetic membrane. Several alternative terms for this technology exist and are often used interchangeably (99), such as extracorporeal life support (ECLS), cardiopulmonary support (CPS), and extracorporeal cardiopulmonary resuscitation (ECPR). A particular pumpless setup is termed pumpless extracorporeal lung assist (PECLA) or interventional lung assist (iLA) (Figure 2.8) (100).

Depending on the condition of the patient, the setup can vary between a number of different modes (101,102). In the veno-arterial (VA) modality, blood is drawn from a vein and infused in an artery (Figure 2.8). This approach can be used when circulation or both circulation and respiration are insufficient. In the veno-venous (VV) modality, blood is both drawn and infused into a vein (Figure 2.8). Using dual lumen cannula, this setup can be applied to a single vein. VV type ECMO is used when respiratory impairment is the only indication. Finally, a pumpless modality exists that takes advantage of the natural arterial-venous pressure gradient to drive blood though an extracorporeal oxygenator. By definition, this modality requires sufficient cardiac function to be effective. In rare cases, some of the above modalities have been mixed (103).

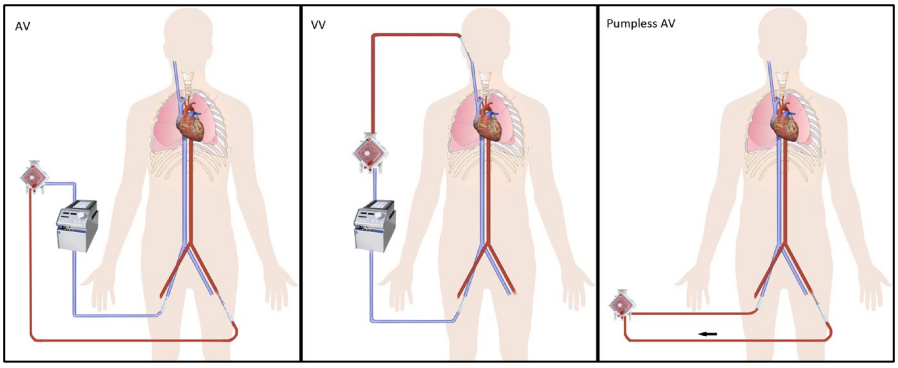

Figure 2.8. The three most common modes of extracorporeal circulation: veno-arterial (VA) mode, veno-venous (VV) mode and pumpless setup.

Extracorporeal circulation has evolved substantially over the last century. Since the early animal experiments using bubble oxygenators, the technique has advanced over film and rotating disc oxygenation into the membrane oxygenators used today,

which are actually based on hollow fibre mats (2,104). The continuous improvements in the performance of the oxygenators is illustrated in Figure 2.9. Simultaneously, innovations in technology regarding pumps, cannulas and the introduction of heparin-coated circuits have also contributed to the improvements in extracorporeal treatment (105).

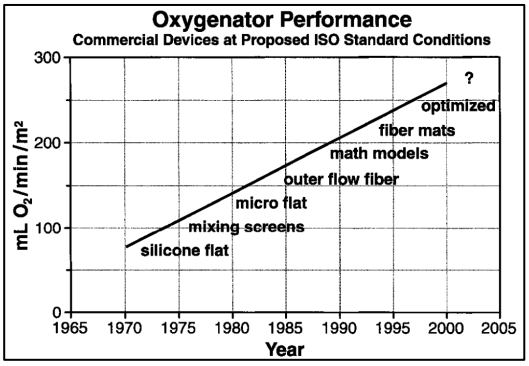

Figure 2.9. Improvements in the efficiency of oxygenators over time. The Y-axis shows the oxygen transfer per area per min. (2)

Gibbon showcased the potential of extracorporeal oxygenation when he performed the first open heart procedure in 1953 to close an atrial septal defect (106). Since then, the use of extracorporeal circulation has become a standard procedure in open heart surgery. However, the first randomized controlled trial from 1979 did not recommend the use of ECMO for respiratory deficiencies (107). In the CESAR trial from 2008, conventional ventilator support was compared to ECMO in patients suffering from severe adult respiratory deficiency syndrome (ARDS). Here, a significant reduction in mortality/severe disability was observed. This finding kick-started a noticeable increase in the use of ECMO and in further research regarding the use of ECMO (108). During the H1N1 flu epidemic of 2009-2010, ECMO was used to treat severe respiratory failure. In a meta-analysis from 2013 regarding the utility of ECMO for H1N1-infected patients, the authors concluded that ECMO is feasible and effective (109).

In patients with cardiac failure, ECMO has proven to be an effective tool (110). The Extracorporeal Life Support Organization (ELSO) has collected data regarding the use of ECMO for various purposes since 1990. Of the 15,942 patients treated with ECMO for cardiac failure, 42% survived to discharge/transfer, while 29% of those placed on ECMO for ECPR survived to discharge/transfer (111). In patients with accidental hypothermia and circulatory/respiratory failure, ECMO treatment using the warming capabilities of the extracorporeal circulation has proved effective on several occasions (112–114).

In addition to the risk of infection, which is always present whenever the skin is penetrated, the ECMO treatment is associated with several complications, such as vascular complications, leg ischemia, bleeding, hyper-fibrinolysis, stroke, and air embolism (115).

In the section above, it has been established how ECMO works, including its advantages and disadvantages. In the next section, I will discuss the effectiveness of ECMO as a treatment for CO poisoning.

ECMO USE FOR CO INTOXICATED PATIENTS

Only three case reports regarding the use of ECMO to treat CO poisoning exist. In a case report from 2009, a CO poisoned patient was subjected to treatment with ECMO (116). The intoxication (HbCO=21.1%) led to both respiratory and cardiac failure; thus, HBO treatment was impossible. Despite intubation and ventilation using FiO_2 at 100%, the PaO_2 was only 4.6 kPa. Therefore, VA-ECMO was established and increased the PaO_2 to 40.0 kPa. Three days later, the patient was weaned from ECMO and became stable enough to discharge from the intensive care unit after an additional 2 days. A similar case was described by Teerapuncharoen et al. in 2015 (84). A CO intoxicated (HbCO=13.6%) patient experiencing cardiopulmonary failure underwent VA-ECMO treatment that increased the blood oxygen content from 8.5 kPa (on ventilator, FiO_2=100%) to 40-65 kPa. The patient was discharged 30 days after admission without neurological sequelae and had regained normal lung function after 24 weeks. In a third case, VV-ECMO was performed on a patient suffering from both respiratory and circulatory insufficiency for 7 days (117). This patient also survived without neurological sequelae.

In an animal study using rabbits (118) by Yin et al., $ECMO_3$ (ozone instead of oxygen) exerted better effects than an oxygen treatment, with a faster reduction in HbCO levels.

The case reports along with the animal study described above indicate that ECMO treatment could be advantageous for severe CO poisoning. Nevertheless, additional research to confirm this hypothesis is required before the treatment is generally recommended. One advantage is that mobile ECMO systems have been developed and may be used during transfer of the patient, or the ECMO device can be brought to the patient (119).

In the next section, I will discuss the challenges in the aerial transport of patients, and why ECMO treatment may be beneficial in some cases.

2.4 ECMO FOR TREATMENT DURING FIXED WING AIRBORNE TRANSPORTATION

CHALLENGES IN THE AERIAL TRANSPORT OF PATIENTS

Occasionally, long-distance airborne transfer of patients is required when injured/ill patients are located in isolated geographical locations or when multiple casualties occur and local medical resources are depleted. Shorter distances can be covered using rotary wing aircraft (helicopter), but for long distances, fixed wing aircrafts (planes) are more efficient due to the higher speed and longer range of travel (120). Nonetheless, airborne transportation using a fixed wing aircraft can be challenging. The aircraft cabin, particularly in military aircrafts, represents a hostile environment for the patient and the crew (121). Furthermore, medical equipment, i.e., ventilators, may perform differently than at sea level conditions (122). Another factor that may influence medical equipment is vibrations, which habitually accompany airborne activities and may cause failure or malfunction. Vibrations have also been shown to increase muscle activity and thus oxygen consumption (123). Noise inhibits free communication and masks sound alarms that are capable of alerting medical personnel (124). Gravitational forces (G-forces) should be considered when placing the patient inside the cabin as the pooling of blood in the opposite direction of acceleration caused by G-forces (125) may exert devastating effects on the patient. However, the primary complicating factor is the decrease in air pressure that occurs with increasing altitude, as described by Boyle´s Law from 1662: $P_1V_1=P_2V_2$. This decrease in air pressure leads to a corresponding reduction in the oxygen content. Most aircrafts prevent a decrease in air pressure by pressurizing the cabin, although the maximum level of pressurization is typically approximately 80% of sea level pressure and equivalent to an altitude of 8000 feet (ft) (126). Weather, air traffic and fuel consumption are important factors (127), and if turbulence is a concern, altitudes of approximately 8,000-10,000 ft. are associated with less turbulence (128). When conducting a military aerial MEDEVAC, tactical considerations may take priority over the condition of the patient/staff. Altogether, the aforementioned factors may limit the possibilities of conducting an airborne MEDEVAC for critically ill patients (129).

The Royal Danish Air Force (RDAF) possesses custom-made intensive care MEDEVAC modules that can be loaded into the cargo bay of the Hercules C130J aircraft. These modules limit the impacts of noise and vibration and additionally create a temperature-controlled, lighted and air conditioned environment for the medical staff and the patient (Fig 2.10). Nevertheless, the limitations of reduced pressure with increasing altitude and the impact of G-forces remain the same. We lack knowledge regarding the use of ECMO in these modules.

In the next section, I will discuss how ECMO has previously been used during fixed wing aircraft transport of patients.

Figure 2.10. Loading of an intensive care module (MEDEVAC module) into a Hercules C-130J Aircraft (left image). The right images shows the interior of the module with patient bays along the walls. (Study 3)

ECMO DURING THE TRANSFER OF PATIENTS USING FIXED WING AIRCRAFTS

Several studies examining the use the use of both VV-ECMO and VA-ECMO during fixed wing flights have been published (130,131). The most comprehensive study regarding transfer of patients while on ECMO was published in in 2014 by Bryner et al. (132). However, of the 221 patients, only 25 were transported by fixed wing aircrafts, with the furthest transfer reaching 3,100 km. The only fatality among all 221 patients occurred while the staff were preparing for takeoff prior to a fixed wing transfer. In a new French retrospective study published in 2018, the authors describe 19 patients who were transferred from Reunion Island to Paris (approx. 10,000 km) for a heart transplant while undergoing treatment with VA-ECMO during the flight (133). No fatalities occurred during these long-distance transfers. In a military setting, Fang et al. evaluated the efforts of the Landstuhl Acute Lung Rescue Team (ALERT), a team undertaking the aerial transfer of military casualties suffering from severe pulmonary insufficiency and in need of extracorporeal assistance (119). Three patients were moved by fixed wing aircrafts while being subjected to extracorporeal support; one on pumpless (PECLA) support and two on VV-ECMO. The feasibility of long-distance PECLA had previously been reported in a porcine study by Kjærgaard et al. (134).

None of the aforementioned publications initiated ECMO as a consequence of CO intoxication. Furthermore, in all cases, cannulation and the initiation of ECMO were completed prior to transfer. Accordingly, we lack knowledge regarding the in-flight use of ECMO for CO poisoned patients and the possibilities of performing cannulation while airborne. Several factors contribute to the complexity of

performing this surgical procedure during flight, from connectivity, compatibility and fastening of medical equipment to the MEDEVAC module, ergonomics of the patient bay, to the safety of the medical staff performing the procedure. All these factors must be tested before medical staff can consider performing this surgery in a real MEDEVAC scenario.

3 AIMS AND HYPOTHESIS

The general aim of this thesis was to obtain insights into the epidemiology of CO-poisoning in Denmark and to develop a novel approach for the treatment of severely CO intoxicated patients using ECMO. Additionally, the feasibility of applying this ECMO treatment during fixed wing aerial transport of patients with severe CO intoxication was explored. Three studies were designed to achieve all of these goals.

The aims of the individual studies were:

1) To clarify the extent of CO poisoning, its mortality and factors contributing to mortality.

2) To investigate the therapeutic effect of VA-ECMO following severe CO-poisoning in an experimental porcine model. We hypothesized that VA-ECMO would improve survival.

3) To demonstrate the feasibility of using ECMO as a potential en route therapy following severe CO poisoning in a porcine model and to explore the possibilities for VA cannulation and initiation of this treatment in flight.

4 MATERIALS AND METHODS

In this section, I will introduce the registries used in Study 1. I will briefly describe the materials and methods used in all three studies included in this thesis. The methodologies of the individual studies are described in detail in the individual papers. Three studies were included in this thesis; one retrospective registry-based epidemiological study and two clinical experimental studies using a porcine model.

4.1 STATISTICAL ANALYSES

For data management in Study 1, the statistical software program SAS (version 9.4, SAS Institute, Cary, North Carolina, USA) was used. For the analyses in all three studies, the statistical software R (version 3.4.1) combined with RStudio (version 1.0.153) were used.

A chi^2-test was utilized to evaluate differences between subgroups in the study population investigated in study 1 and an unpaired t-test was used to compare differences between treatment groups in Study 2. In Study 1, a Kaplan-Meier survival analysis was conducted along with the construction of a Cox regression model. We excluded data from patients who died within the first 30 days of CO exposure to reduce the selection bias introduced when deciding which patients were eligible for HBO treatment.

The resource equation was used (E=total number of animals-number of treatment groups) to calculate an appropriate sample size for Study 2 (135,136). E between 10 and 20 is considered sufficient. Adhering to the "Three Rs" ethical guidelines for animal research (137), E was set to 10; the total number of animals was 12, with 6 in each treatment group. A paired sample t-test was used when comparing the mean PaO_2 at baseline with the mean PaO_2 at time of cardiac failure. Regression analyses were performed to predict PaO_2, PVR and lactate levels from HbCO levels.

No specific statistical analyses were performed in Study 3.

4.2 STUDY 1

Study 1 was a retrospective observational study that compiled data from several databases in Statistics Denmark (approval: GEH-2014-013 I-Suite nr: 02731). When conducting registry-based studies in Denmark, researchers are not required to

obtain approval from an ethical committee or from the individuals included in the study.

We collected data from 01.01.1995-31.12.2015. Prior to this time period, ICD coding was different and data from the National Prescription Registry were not available. The date of 31.12.2015 represents the latest date with completely updated registers.

People living in Denmark are included in The Danish Civil Registration System (CRS), which was founded in 1968 (138,139). Each person is given an individual Civil Personal Register (CPR) identification number, which is used for all contacts with the public administration, including health services. This personal number can be used to blend information from numerous registries; however, as it is encrypted, the identification of an individual is not possible.

THE DANISH NATIONAL PATIENT REGISTRY

The Danish National Patient Registry (DNPR) contains information about contacts with hospitals and emergency rooms from 1978 to the present (140). Information regarding the character of the admission are logged, along with information regarding the diagnosis relevant to the current admission and procedures performed (including hyperbaric oxygen therapy). From 1994 to the present, this information was coded according to the WHO International Classification of Diseases version 10, ICD-10 (141).

THE NATIONAL PRESCRIPTION REGISTRY

Since 1994, the information about all drugs sold based on prescriptions in Denmark have been collected in this registry (142). In study 1, this registry was solely used to identify patients suffering from insulin-dependent diabetes.

THE DANISH REGISTER OF CAUSES OF DEATH

The Danish register of causes of death is very old, as the collection of information about the causes of death was initiated in 1875 (143). From 1970 to the present, the information has been registered in an electronic database using the ICD system, similar to the DNPR. The cause of death registry contains information about the time and place of death, manner of death, main cause of death and factors contributing

to death. The information is entered by the hospital doctor, the forensic medical examiner or the patient's own general practitioner.

The compilation of data from the registries mentioned above, based on a diagnosis of CO poisoning/smoke poisoning, was performed along with statistical analyses based on these data.

4.3 STUDIES 2 AND 3

Both Studies 2 and 3 were approved by the Danish Animal Experiments Inspectorate J.nr. 2016-15-0201-01064. After completing the experimental protocol, all animals were sacrificed by an injection of a lethal dose of pentobarbital.

The use of a CO alarm was enforced in all studies involving CO gas to secure sufficient warning in case of a leak. Cardiac failure was defined as a decrease in cardiac output of 50%, and cardiac arrest was defined as a systolic blood pressure less than 25 mmHg. In Study 2, the primary outcome was survival in the absence of extracorporeal circulation for at least 10 minutes without cardiopulmonary collapse. Secondary outcomes were changes in pulmonary vascular resistance (PVR) and damage to the lung tissue.

Twelve female pigs (mean weight 48 kg) were included in Study 2. For an illustration of the setup, see Figure 4.2, and a picture of the actual setup is presented in Figure 4.3. A similar setup was used in the Hangar at Aalborg Airbase in Study 3. Animals were poisoned until cardiac failure occurred. At this time point, the allocation to either ventilator treatment using an FiO_2 of 100% or ECMO was revealed. If cardiac arrest occurred, it was treated using the 2015 guidelines from European Resuscitation Council (144). If cardiac arrest developed in the ventilator group and if no signs of improvement in the condition occurred within 10 min of resuscitation, then the ECMO treatment was also initiated in this group (Figure 4.1).

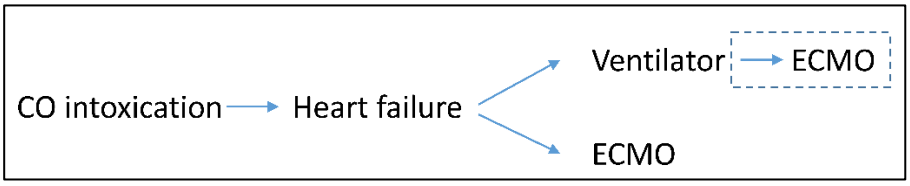

Figure 4.1. Flow diagram of the experimental protocol.

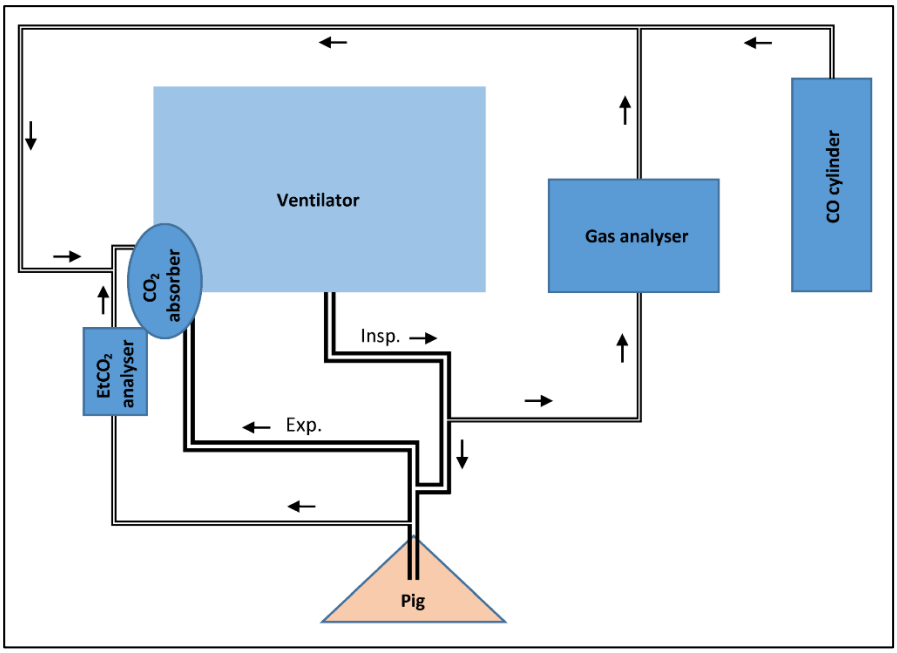

Figure 4.2. Illustration of the flow of air/CO between the equipment and the research animal.

Samples of lung tissue were obtained at baseline and at time of randomization. These samples were preserved in formalin and transported to the Department of Forensic Medicine, Aarhus University for histological analyses using basic haematoxylin & eosin (H&E) staining.

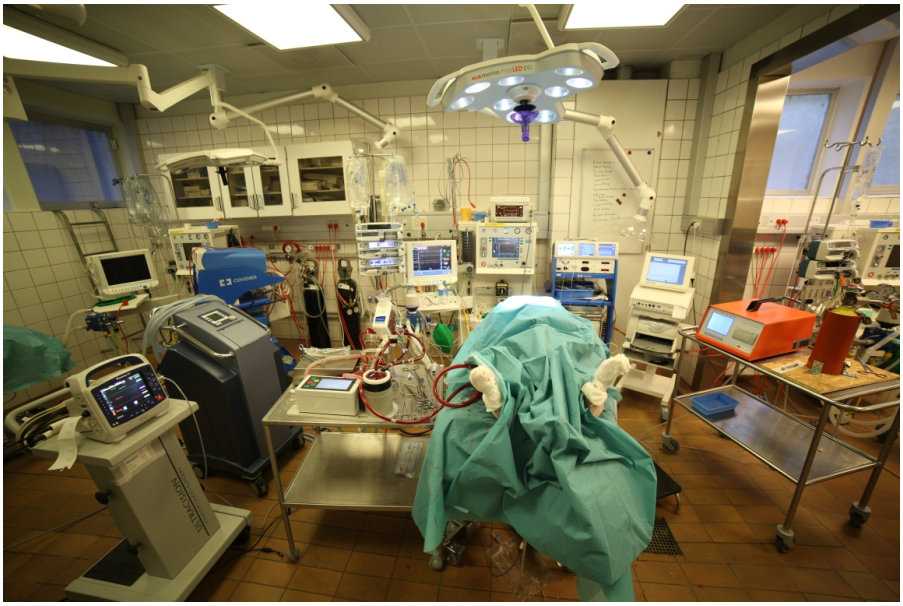

Figure 4.3. Experimental setup.

In study 3, one female pig (Danish Landrace) weighing 39 kg was used. For the collection of arterial blood gas samples, we used a mobile unit (ABL90 FLEX-Series, Radiometer Medical, Brønshøj, Denmark) that was operated by technicians from Radiometer, Denmark. A sheath was placed in the femoral artery on the left side and a sheath was placed in the femoral vein on the right side.

The ECMO setup consisted of a centrifugal pump (Rotaflow, Maquet, Rastatt, Germany), an oxygenator (QUADROX adult, Maquet, Rastatt, Germany), a 19 French venous Cannula (Medtronic, Minneapolis, Minnesota, US) and a 15 French arterial cannula (Novaport, Novalung, Heilbronn, Germany). During the treatment with ECMO, the oxygen flow was set to a rate of 2 L/min. The flow rate of blood through the external circulation was approximately 2 L/min.

An ambulance from the Air Force transported the pig from the Biomedical Research Laboratory to a hangar at Aalborg Air Base. The pig was the poisoned using the same methods as described in study 2. Then, the pig was transferred to the aircraft (Hercules C-130J, Lockheed Martin, Bethesda, Maryland, USA) and placed inside the MEDEVAC module (Figure 4.4). Cardiac arrest was induced at an altitude of 8,000 ft. Then, chest compressions were performed while both arterial and venous cannula were placed using the Seldinger method via the sheaths that had previously been placed in the femoral blood vessels (Figure 4.4). ECMO was initiated once the cannulas were in place. Resuscitation continued with use of DC-shocks (Zoll Pro Pac MD, ZOLL Medical Corporation, Chelmsford, Massachusetts, US) and medication, in

accordance with 2015 guidelines from the European Resuscitation Council (144). Along the flight path, a short decrease in altitude to 2,500 ft was required for reasons not related to the experiment. Cardiac arrest was induced 50 minutes into the flight, and the total duration of the flight was two hours where, after which the aircraft returned to Aalborg Air Base and the pig was returned to the hangar. After completing the experiment, the pig was euthanized with an intravenous injection of a fatal dose of pentobarbital.

Figure 4.4. The pig was placed in the centre of the module to enable access from all sides. Cannulation was performed during cardiac arrest and chest compressions. (Study 3)

5 RESULTS

In the sections below, I will present the results of each study. Further results are presented in the individual papers.

5.1 STUDY 1

In this study, a total of 22,930 patients suffering from CO intoxication were included. The total number of cases per year along with corresponding number of fatalities are illustrated in Figure 5.1. Corresponding rates are illustrated in Figure 1 of Study 1. Of the total patients, 1,792 (8%) were declared dead prior to hospitalization and 21,138 (92%) patients were hospitalized. The total 30-day mortality rate was 9.2% (n=2,102). Nevertheless, the 30-day mortality rate was only 1.5% (n=310) among the patients who made it to a hospital alive. The median follow up time was 8.1 years.

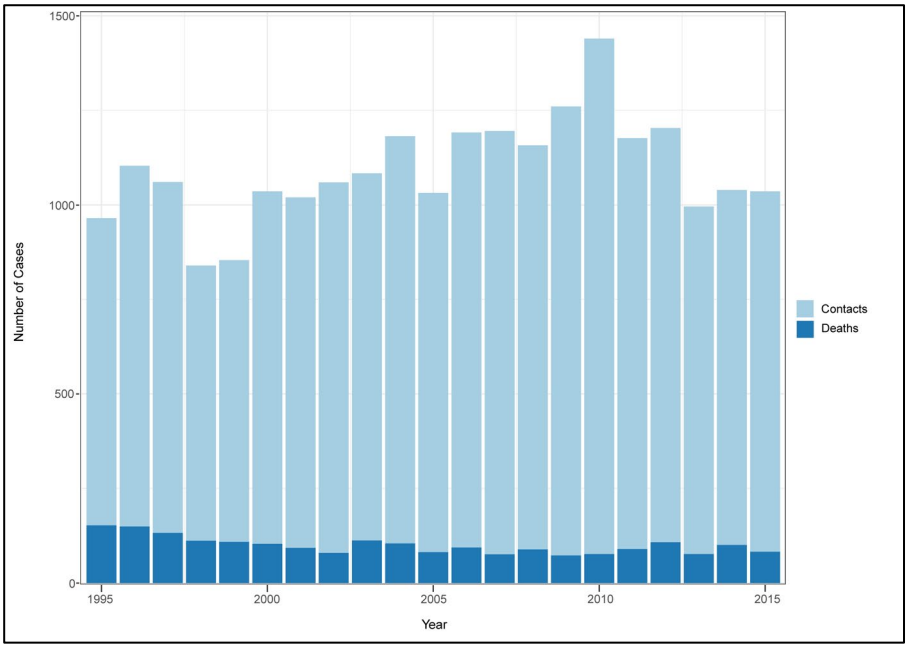

Figure 5.1. Overview of the total number of patients (light blue) and number of deaths per year (dark blue). Total number of cases= 22,930.

Information about the manner of death was extracted for 1,957 patients. Subjects who committed suicide preferred using gas and not fire/smoke; of the 1,957 deaths,

1,132 deaths were caused by gas/vapours, with 91.1% of cases being intentional. Of the 1,957 deaths, 825 deaths were due to fire smoke, with only 7.2% of these being intentional.

In the survival analysis, plots of the survival of patients who were hospitalized and survived for more than 30 days after CO poisoning were drawn. The patients were divided into two groups stratified by the administration of HBO treatment (Figure 5.2). This analysis showed diverging survival lines that were significant at the 95% confidence interval after six years (p<0.001).

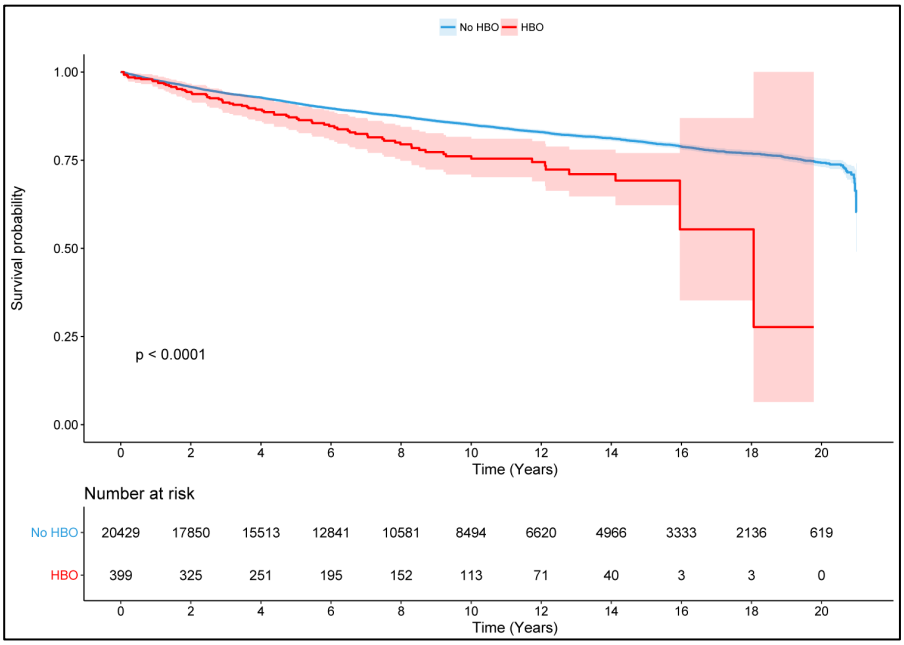

Fig 5.2. Kaplan Meier plot of survival in groups stratified by treatment. Only patients who survived for more than 30 days were included. Light blue-/red areas depict 95% CIs. (Study 1)

A forest plot of the Cox regression model is shown below (Figure 5.3). After adjusting for the effect of co-morbidities, the HBO treatment was no longer associated with increased mortality (HR=1.2, p=0.14). This trend was also observed for drug abuse (HR=1.1, p=0.35).

Increasing age was strongly correlated with an increased risk of mortality in age group 3 (HR=3.2, p<0.001) and age group 4 (HR=15.5, p<0.001). Prior co-morbidities that were associated with increased mortality were alcohol abuse (HR=2.1, p<0.001), psychiatric disease (HR=1.5, p<0.001), arterial embolism (HR=1.5, p=0.01), COPD (HR=1.7, p<0.001), cerebrovascular disease (HR=1.5, p<0.001) and atrial fibrillation

(HR=2.3, p<0.001). Drug abuse (HR=1.1, p=0.35), stroke (HR=1.1, p=0.40), and gender (HR=0.96, p=0.32) were not significant predictors of mortality.

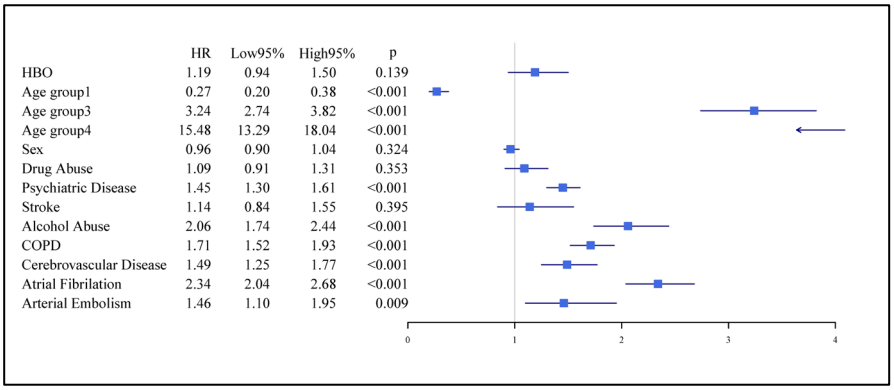

Fig 5.3. Forest plot of the Cox proportional hazards model. Age group1=0-24.4 years, age group2 (reference) =24.4-36.6 years, age group3=36.6-50.75 years, and age group 4=50.8+ years. COPD=chronic obstructive pulmonary disease. Lines illustrate 95% CIs. (Study 1)

5.2 STUDY 2

Only one of the six CO intoxicated pigs that were randomized to ventilator treatment with FiO_2 at 100% survived. Five animals suffered from cardiac arrest (mean of 11.8 minutes after randomization) and a return of spontaneous circulation (ROSC) was not obtained in any of these animals through conventional advanced resuscitation methods. However, after the application of VA-ECMO, ROSC was obtained in four of these animals (80%). All animals in the ECMO group survived, and only one animal suffered from cardiac arrest, however in this case, ROSC was obtained after 17 minutes (Figure 5.4, Study 2).

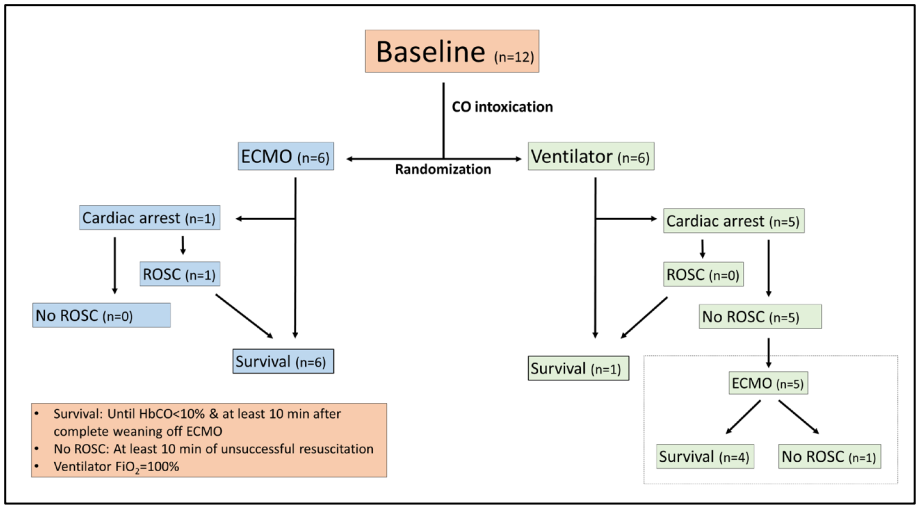

Figure 5.4. Flow chart of outcomes. ECMO=extra corporeal membrane oxygenation, ROSC=return of spontaneous circulation. (Study 2)

The mean time on extracorporeal circulation was 182.5 minutes (SD=63.5) in the ECMO group compared with 201.6 minutes (SD=63.5) in the ventilator group p=0.96.

A significantly higher peak PaO_2 was achieved in the ECMO group compared to the ventilator group (Figure 5.5, Study 2), p<0.001.

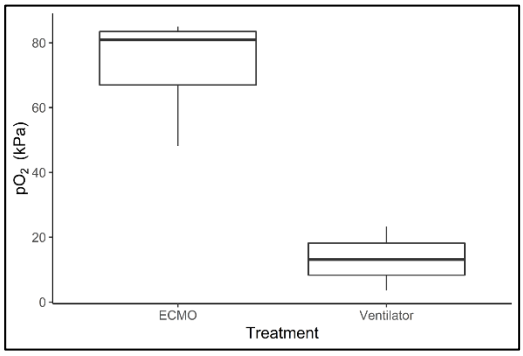

Figure 5.5. Peak arterial pO_2 values after treatment with extracorporeal membrane oxygenation (ECMO) or ventilator treatment was initiated. (Study 2)

When examining the HbCO half-life ($T_{1/2}$), no differences between groups were identified (Figure 5.6). Overall, the $T_{1/2}$(HbCO) was 77.2 minutes (min), with a $T_{1/2}$(HbCO) for the ECMO group of 86.0 min, and $T_{1/2}$(HbCO) for the ventilator group of 70.0 min. This analysis is not included in Study 2. No significant difference was observed in the mean time required for HbCO levels to decrease to less than 10%

after intoxication: 123.7 minutes (SD=20) in the ECMO group compared with 163.7 minutes (SD=15.2) in the ventilator group, p=0.56.

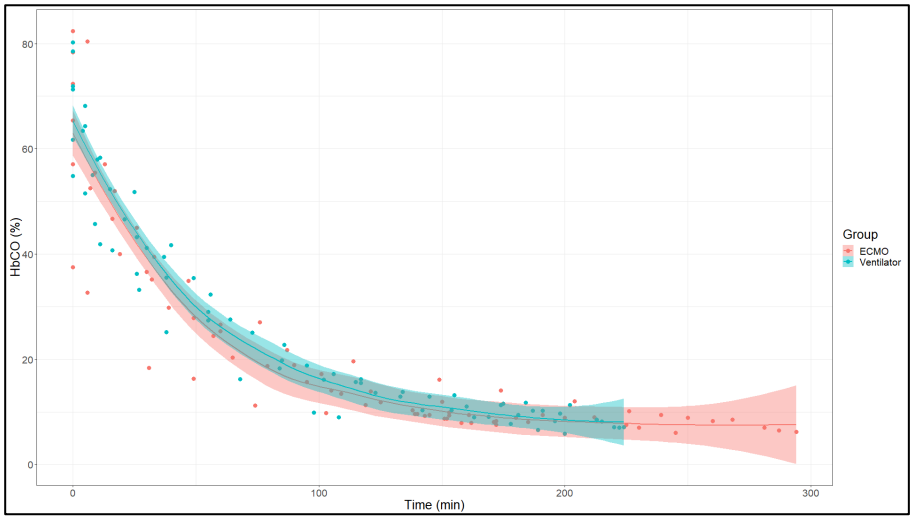

Figure 5.6. Decrease in haemoglobin-CO (HbCO) levels over time in each treatment group. Light-coloured areas depict 95% CIs. ECMO= extracorporeal membrane circulation.

Consistent changes were not observed in a comparison of lung tissue samples obtained at baseline and after severe CO intoxication (time of randomization). Fluid did not accumulate in the alveoli and inflammation was not detected. Figure 5.7 illustrates the histology before/after CO poisoning.

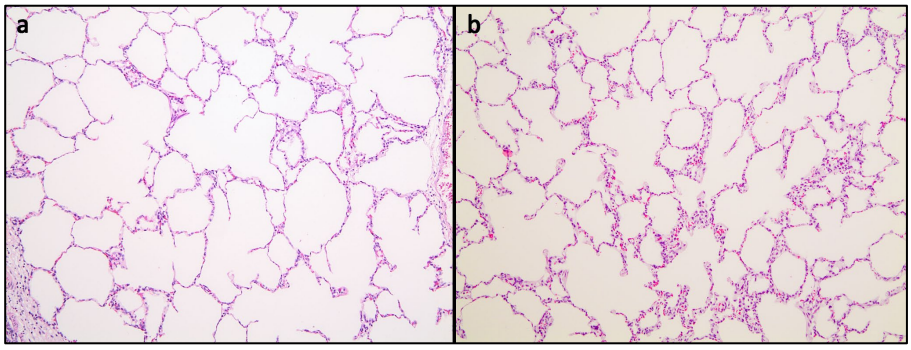

Figure 5.7. a) Haematoxylin & eosin-stained tissue samples obtained prior to CO intoxication (baseline, HbCO=3.6%) and b) at the time of randomization (HbCO=71.1%). Both images were captured at 100 X magnification.

5.3 STUDY 3

Cannulation was successfully performed at 8,000 ft during cardiac arrest and followed by VA-ECMO treatment (Figures 5.8. and 5.9). The pulse generating sinus rhythm was restored upon defibrillation 29 min after cardiac arrest. Complete weaning was performed once on the ground again after being airborne for 127 min. Figure 5.8 depicts major events along with altitude, and Table 5.1 lists corresponding arterial blood gas values. Oxygenation during both ventilator treatment and VA-ECMO treatment was high at 56-82 kPa and 40-58 kPa, respectively.

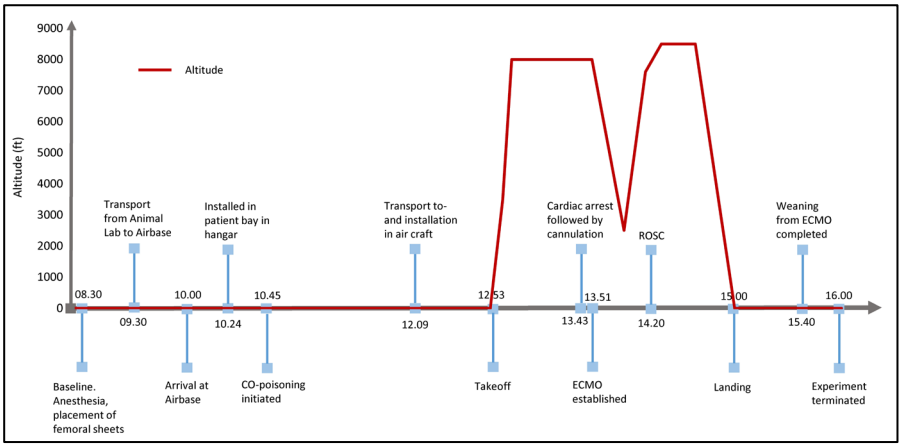

Figure 5.8. Significant events are plotted over time and the corresponding altitude is represented with the red line. Arterial blood gas samples are listed in Table 5.2. Abbreviations: extracorporeal membrane oxygenation (ECMO) and return of spontaneous circulation (ROSC). (Study 3)

Figure 5.9. Cannulation was performed and ECMO treatment was established.

Time	Event	Alt. (ft)	pH	$CO_{2\ (Kpa)}$	$pO_{2\ (Kpa)}$	Lactate	HbCO (%)	BP (mmHg)	HR
08.33	Baseline	0	7.46	4.77	42.8	0.8	0.3	104/58	51
10.24	Transfer to Hangar completed	0	7.5	5.04	52	0.7	0.3	100/54	52
10.35	Arterial blood gas analysis	0	7.48	5.16	37.9	0.7	0.3	97/54	50
10.45	CO-poisoning initiated	0	7.48	5.15	39.7	0.6	14.6	106/52	63
11.05	Arterial blood gas analysis	0	7.46	5.52	40.4	1.1	49.3	144/85	70
11.15	Arterial blood gas analysis	0	7.45	5.53	39.6	1.2	49.0	136/62	70
11.48	Arterial blood gas analysis	0	7.46	5.28	40.4	1.1	58.7	114/62	68
12.09	Transfer to aircraft	0							
12.20	Arterial blood gas analysis	0	7.54	3.83	50.9	1	27.5	87/47	60
12.53	Takeoff	0							
13.05	Arterial blood gas analysis	8000	7.53	4.3	9.19	0.8	12.5	93/47	46
13.06	Ventilator set at 100% oxygen	8000							
13.12	Arterial blood gas analysis	8000	7.49	4.52	57.8	0.7	11.1	95/46	45
13.25	Arterial blood gas analysis	8000	7.46	4.28	59.2	0.7	9.3	113/46	44
13.43	Cardiac arrest induced	8000						21/18	135
13.51	ECMO established	8000						76/74	VF
14.00	Arterial blood gas analysis	4000	7.31	4.4	71.4	4.1	10.9	84/82	VF
14.08	2500 ft 50 mmol K	2500	7.28	4.46	82.1	5.5	9.9	65/62	VF
14.20	ROSC	4500							SR
14.29	Arterial blood gas analysis	8500	7.25	4.72	55.9	7.9	7.3		62
14.38	Arterial blood gas analysis	8500	7.37	3.39	62	6.9	7.5		51
15.00	Wheels on ground	0	7.24	4.63	75.7	8	5.3	100/68	43
15.30	Arterial blood gas analysis	0	7.11	7.41	24.9	6.8	4.7	183/43	62
15.37	Weaning from ECMO initiated	0	7.18	5.59	62.6	6.9	4.5	181/133	112
15.40	Weaning completed	0							
15.45	Arterial blood gas analysis	0	7.15	6.62	24.6	5.7	4.4	123/70	82

Table 5.1. Major events, altitude, vital values and arterial blood gas results obtained during the experiment. (Study 3)

6 DISCUSSION

Humans have suffered from CO poisoning since the discovery of fire. Additional sources of CO intoxication have emerged throughout history, and CO poisoning is now the most frequent form of poisoning in the US (19). Neurological sequelae and a high risk of mortality in patients with severe poisoning have inspired numerous studies in the field of CO poisoning. Despite this research has identified several toxic effects in addition to the strong binding of CO to Hb (75), these findings have yet to be utilized to develop new treatment strategies. Unknown factors contributing to the toxicity of CO may yet be discovered. Oxygen treatment has been the cornerstone treatment for CO poisoning over the last 50-60 years, and both NBO treatment and HBO treatment have been used (22). When oxygen is administered as HBO therapy, several limiting issues are introduced that potentially create a selection bias among patients who are treated.

The aim of this PhD thesis was to investigate the extent of carbon monoxide poisoning in Denmark and important factors that may contribute to mortality. In addition, we also examined the effects of HBO therapy on survival. Furthermore, we introduced a systematic examination of the potential benefits of using extracorporeal circulation to treat severe CO poisoning with subsequent cardiac failure, and finally, we explored whether this approach could be utilized during a long-distance flight in a fixed wing aircraft.

The main findings of study 1 were that CO poisoning is a frequent event that causes approximately 1000 contacts with the hospitals each year and results in approximately 100 deaths, of which 52% are caused by suicide. Several co-morbidities, i.e., alcohol abuse, psychiatric diseases and atrial fibrillation along with increasing age, were associated with increased mortality. The data did not support the hypothesis that HBO treatment would result in improved survival after considering co-morbidities. In study 2, a markedly increased survival was observed when ECMO was administered to treat cardiogenic shock following CO poisoning. An increasing HbCO level was not associated with an increasing PVR, although it was associated with a decrease in PaO_2. No changes were observed in the lung tissue samples obtained during various stages of CO poisoning. In study 3, the same ECMO treatment methods used in study 2 were applied to a CO poisoned pig during a flight in a fixed wing aircraft. Additionally, we successfully performed cannulation and established ECMO treatment while airborne.

6.1 EPIDEMIOLOGY OF CO POISONING

In study 1, 10.8% of all patients included in this study had a prior diagnosis of alcohol abuse, and 8.6% had a previous diagnosis of psychiatric disease. Corresponding percentages from a study performed in Taiwan were 3.7% for alcohol abuse and 32.1% for mental disorders (91). Large differences between our findings and the findings of the Taiwanese study are evident and may be based on differences in the registration of co-morbidities. The data included in the Taiwanese study utilized ICD-9 coding (vs. ICD 10 coding in Study 1) and included "dependence of psychoactive substances" in the "mental disorder" group, whereas these patients were placed in the "drug abuse group" in the data described in Study 1. In addition, the diagnosis of the patient, along with the co-morbidities, may differ from Danish conditions. Other explanations for the discrepancies are differences in the health care infrastructure, source of exposure and severity of exposure. A study from Wuhan, China investigating the manner of death in 156 patients with CO poisoning reported the 33% of these deaths were intentional, and the preferred source of exposure was from charcoal burning/gas installations (20). These findings are consistent with the findings from Study 1, in which the preferred method of committing suicide by CO poisoning was gas exposure (exposure 95%) and not fire-related exposure. The Taiwanese study reported a suicide percentage of 20.4; however, this number includes both suicides and attempts. In Study 1, 5.7% of the entire group of CO poisoned patients committed suicide, and 52% of all deaths occurred following intentional poisoning, which is higher than the value reported in Wuhan. Data are not available to investigate the percentage of suicide attempts in Study 1. Another paper by the same authors as the Taiwanese study using the same data examined a range of additional co-morbidities using a Cox proportional hazards regression analysis (73). Here, both similar and divergent results are noticed for alcohol abuse (HR of 2.06 in Study 1 vs. 1.69 in the Taiwanese study), stroke (HR of 1.14 in Study 1 vs. 1.88), COPD (HR of 1.71 in Study 1 vs. 1.01) and psychiatric diseases/mental disorders (HR of 1.45 vs. 1.76). A higher percentage of patients were treated with HBO (28%) in the Taiwanese study than in Study 1 (4.5%). Again, conclusions based on these numbers are difficult to draw, as substantial differences in the methods, population and indications of HBO exist between studies.

6.2 ECMO AS A TREATMENT FOR CO POISONING

ECMO significantly improved the survival of animals with CO poisoning in the experimental porcine model. Potential survival benefits have previously been described in three case reports. In a case report by Wang et al., a 23-year-old male was admitted due to CO poisoning (145). The HbCO level was 21% at time of admission and the patient was semi-comatose. Despite endotracheal intubation and

ventilation using FiO_2 at 100%, the PaO_2 was only 6.0 kPa. These results are consistent with the decrease in PaO_2 observed after CO poisoning (see Figure 2b in Study 2), and thus ventilator treatment may be insufficient. The authors observed "myocardial injury with myocardial suppression" and subsequent pulmonary oedema. VA-ECMO therapy increased the PaO_2 from 4.6 kPa to 40.0 kPa. In our study, a much higher arterial PaO_2 was observed in the ECMO group, and this finding is consistent with the case report. The patient described in the case report was successfully weaned after 3 days on ECMO treatment, but information about the neurological outcome was not provided. In a case report by Teerapuncharoen et al., a woman with CO poisoning exhibited a HbCO level of 13.6% several hours after the initiation of ventilator treatment with FiO_2 at 100% (84). This patient developed circulatory collapse and was too unstable to be transferred to HBO therapy. Instead, VA-ECMO treatment was initiated and stabilized the circulation. The patient was weaned after four days and no neurological deficits were observed. In another case report by McCunn et al., a CO intoxicated victim was found unresponsive/apneic after a fire (117). Although the initially measured HbCO level of 40% decreased to 9% and the patient was supported by a ventilator using an FiO_2 at 100%, the PaO_2 was still very low (4.9 kPa). Since the patient was deemed too unstable to receive HBO treatment, extracorporeal lung assist (PECLA) was initiated and immediately improved the PaO_2 to 12.7 kPa. The patient improved over time and was discharged without neurological impairments. These three case studies illustrate the diverse pathology and symptomatology of CO poisoning. For practical reasons, the pig was weaned from ECMO once the HbCO level was less than 10%. However, based on the clinical case studies, a much longer ECMO treatment is probably be needed.

One of the main concerns regarding the use of ECMO for CO poisoning is the potential for poor neurological outcomes associated with cardiogenic shock and cerebral ischemia. Nevertheless, none of the three cases described above presented neurological deficits after treatment with ECMO. When considering ECMO therapy, the estimated time to the initiation of treatment should be considered, as an increase in the time to the initiation of ECMO is associated with worse neurological outcomes (146). Studies 2 and 3 did not investigate the neurological impacts of CO poisoning, and future studies should consider including this aspect into the protocol.

The improved survival of the ECMO group may be due to the stabilization of the cardiac function, which other studies have reported to be affected by CO poisoning. Thirty-seven percent of patients included in the study by Garg et al. presented with "myocardial injury defined by elevated biomarkers and electrocardiographic (ECG) changes" (147). These findings are supported by Satran et al., who observed ischemic ECG changes in 30% of their patients (148). Two studies observed reversible cardiac stunning/dysfunction following CO poisoning (149,150). Thus, CO poisoning appears to be capable of causing transient cardiac ischemia, which may result in infarction if the CO intoxication is severe or persistent. ECMO treatment may be beneficial in this

regard, as it increases the oxygen content in the blood and minimizes cardiac stress and oxygen consumption through the support of the extracorporeal circulation.

Are other benefits of ECMO treatment identified in this study explained by a reduction in $T_{1/2}$(HbCO) and therefore sooner resolution? We speculated that the ECMO treatment would induce a higher PaO_2 level than the use of pure oxygen administered through the ventilator. This hypothesis appeared to be validated (Figure 5.5, Study 2). However, as ventilator treatment was replaced with ECMO treatment in 83% of patients after conventional resuscitation had failed, may potentially favour ECMO treatment itself. Because the ECMO treatment increased the PaO_2, a reasonable hypothesis is that this change would reduce the $T_{1/2}$(HbCO). However, this hypothesis did not appear to be validated. In study 2, the $T_{1/2}$(HbCO) was 77.2 minutes overall. This value corresponds well with the $T_{1/2}$(HbCO) reported by Weaver et al. after the administration of 100% oxygen under normobaric conditions (151). The ECMO treatment did not exert an apparent beneficial effect on $T_{1/2}$(HbCO) in the present study. However, our study (Study 2) was not specifically designed to investigate $T_{1/2}$(HbCO) in detail. Thus, the ECMO treatment was administered to both groups, and no control group with an FiO_2 of 21% was investigated. To the best of our knowledge, no studies describing $T_{1/2}$(HbCO) during ECMO treatment have been reported. An animal study investigating the use of ozone (O_3) in the extracorporeal circuit instead of oxygen for CO poisoned rabbits observed significantly lower HbCO levels 30 min after CO intoxication compared to the group treated with 100% FiO_2 alone (82). No group was treated with ordinary ECMO in this study. A new, separate study should be designed with the main goal of properly exploring the potential beneficial effects of the ECMO treatment on the $T_{1/2}$(HbCO).

In the biopsies of the lung tissue, we were unable to identify any changes using histological examinations. We observed a decreased PaO_2 and increased HbCO levels (please refer to Study 2, Figure 2b). This finding is consistent with the findings described in the three case reports mentioned above, where only an insufficient PaO_2 was achieved, despite ventilator therapy with an FiO_2 of 100%. This finding might be based on the increase in the diffusion barrier caused by CO poisoning. One cause of an increase in the diffusion barrier is pulmonary oedema. Pulmonary oedema has been reported to be associated with CO poisoning in several studies (152,153). The cause of this association may be thermal injuries (154), backward failure due to depressed cardiac activity, or tissue irritants present in the inhaled smoke, i.e., hydrogen chloride (HCl) (155). However, it cannot be excluded, that CO itself may cause tissue damage in the alveoli. In both studies 1 and 2, pure CO was administered, eliminating the risk of assessing damages caused by factors other than cardiac failure and CO itself. Despite the presence of cardiac failure, oedema was not observed in the tissue samples, potentially due to the short duration of cardiac failure, which was not a sufficient period for oedema to develop. Additionally, no changes in PVR were detected with increasing HbCO levels (Study 2, Figure 2c). Again, this lack of changes might have been due to the relatively short duration of

CO poisoning. Changes in the diffusion barrier, i.e., changes in surfactant levels or basement membrane that could cause diminished diffusion of oxygen (156), may not be detected by the examination of the tissue, as simple H&E staining was used. Moreover, immunological changes might have played a role, as suggested in the study by Pieri et al., who observed increased macrophage activity following CO-poisoning (157). These changes would not have been detected in the histological evaluation. Examinations using electron microscopy might have provided further insights.

As we did not identify a correlation between increasing PVR and HbCO levels, Study 2 does not support the hypothesis that cardiac failure, in cases of CO poisoning, is caused by increased PVR and subsequent backward failure. This finding is consistent with the results from a study by Zyckerbraun et al., who found that a low dose of CO reversed pulmonary arterial hypertension in rats (55).

Several studies have reported the airborne transport of patients undergoing ECMO treatment using VV-ECMO, AV-ECMO and PECLA (119,133). However, during the literature review, studies reporting cannulation being performed while airborne were not identified.

We chose to perform the feasibility test using a real aircraft that was actually flying during the experiment. In our opinion, this approach provided a realistic impression of the challenges associated with performing a flight with a real CO intoxicated patient. Another option would have been to use a hypobaric chamber to simulate the pressure conditions of the flight. This approach has previously been employed by Kjærgaard et al., and in a paper published in 2007, the authors described the use of interventional lung assist (iLA) during different modes of transportation, including fixed wing aircraft, and tests in a hypobaric chamber (158). The authors observed a decrease in PaO_2 with increasing altitude, both real and simulated. This decrease is consistent with the findings from Study 3, as a clear increase in PaO_2 was observed during a period of a decreased flying altitude (Study 3, Table 1, times: 14.00 and 14.08). The authors repeated the experiment during an intercontinental flight once testing in the hypobaric chamber had been performed. The use of a hypobaric chamber has some advantages, as it is less costly, requires less logistic planning and the pressure can be controlled precisely for long periods of time. Nonetheless, factors that may complicate a flight, such as turbulence, G-forces and vibration, cannot be recreated, and the impact on both medical personnel and the patient can therefore not be tested. Additionally, the compatibility of the medical equipment with the aircraft is an important factor when evaluating feasibility and should therefore be tested during a real flight.

With the exception of the aforementioned study, we did not identify papers describing the use of ECMO in a hypobaric chamber in either a clinical setting or in

an experimental setup. In addition, we were unable to identify any published papers examining the use of ECMO in a hyperbaric chamber.

In Denmark, the Air Force has four intensive care MEDEVAC modules at its disposal (Figure 6.1), which are routinely used during aeromedical evacuation flights.

Figure 6.1. Intensive care MEDEVAC module - exterior and interior (photos courtesy of Jean-Michel Ferrieux).

These modules are prototype models that are not available in any other NATO country. Many countries within NATO have expanded their own capacity using many different approaches. The most common method is to install stretchers and medical equipment directly in the cargo bay of the aircraft (Figure 6.2), whereas a few other countries use the modular approach (159). No published overview on the MEDEVAC capabilities of the individual NATO countries exists. Advantages and disadvantages exist for both types of approaches. Modules create better environment for the patient and the staff; however, the loading and unloading of the modules makes the aircraft less flexible for use in other operational configurations. Additionally, MEDEVAC modules take up a large percentage of the cargo bay, and the total capacity of transporting patients is therefore diminished. Nevertheless, as described in chapter 2.4 of this thesis, the MEDEVAC modules have substantial advantages compared to the cargo bay when transporting a critically ill patient, both for the patient and the personnel.

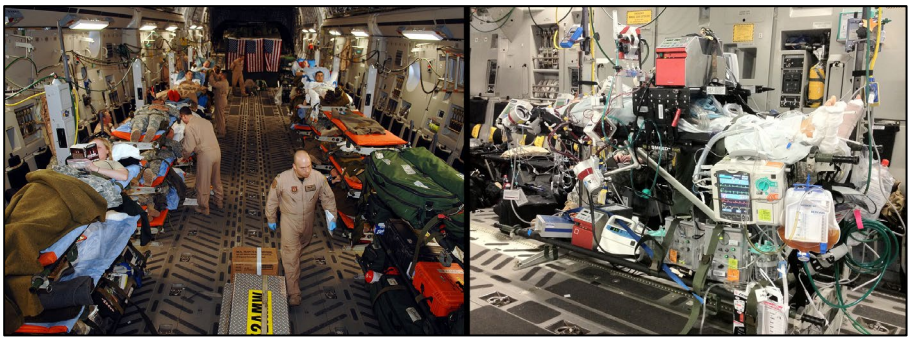

Figure 6.2. The left image shows the interior of a US C-17 aircraft during an aeromedical evacuation of patients from Iraq to Germany. The right image shows a patient on veno-venous extracorporeal circulation transported in a C-17 aircraft from Japan to Hawaii.

6.3 STRENGHTS AND LIMITATION OF THE STUDIES INCLUDED IN THIS THESIS

A strength of study 1 is that a large number of patients, approximately 23,000 persons, were included, and the median follow up time was relatively long, 8.1 years. Notably, Denmark is a very homogenous country, and all people living in Denmark have equal access to health care services, adding to the credibility of the registries used in this study, as it limits the potential for a socioeconomic bias. Moreover, the validity of the registries from Statistics Denmark used in the present study is considered high (140,160–163). Another strength of study 1 is that several co-morbidities were included in the statistical models and actually changed the outcome of the comparison between patients who did or did not receive HBO treatment. In the Kaplan-Maier plot, a statistically significant difference was observed between the survival curves (Figure 4, Study 1); however, this difference disappeared after adjustment for co-morbidities in the Cox model (Figure 5, Study 1).

One of the main limitations of study 1 is that it was a registry-based retrospective study, and therefore we were unable to draw any conclusions regarding causality of the factors investigated, but only described associations. Another limitation of this study is the lack of clinical data for HbCO values, lactate levels, electrolyte levels, cardiac rhythm, and blood pressure. The inclusion of these data in the Cox model might have increased the accuracy of our estimates, which might have contributed to a better understanding of how these clinical parameters influence mortality and morbidity. Unfortunately, this information was not accessible. In addition, in the statistical models, we were unable to ensure that other confounding factors, not included/unknown, might have had influenced the results.

Studies 2 and 3 shares some strengths and limitations regarding the use of animals as substitute for humans in research. The main limitation of animal studies is the interpretation of how the results may translate to a clinical setting with human patients. We surmised that the use of a large animal model would provide us with results that are more translatable to humans than a model using rodents. We chose a porcine model for several reasons. Pigs are similar in size to humans and have a comparable anatomy and cardiac/pulmonary function (respiratory frequency, heart rate, blood pressure, arterial blood gas values, etc.) (164). The same instrumentation and monitoring devices can be used on animals and humans, which is an important factor when evaluating the feasibility of the experiments. Additionally, pigs have previously been used in studies exploring different aspects of applying ECMO technology to treat different conditions (165,166). However, some differences that may affect translation to human conditions must be considered. For example, a pre-existing collateral coronary blood supply is not present in the pig heart (164).

A comparison of the ECMO treatment with a group subjected to HBO treatment would have been valuable. However, this comparison was not possible considering the distance to the nearest hyperbaric chamber. For this reason, the results cannot be directly compared with results that would potentially be obtained when administering HBO therapy.

Another major limitation is that neurological outcomes were not evaluated because biomarkers of neurological ischemia were not measured during the experiments and the animals were euthanized at the end of the experiments.

In study 3, the pigs were placed on ECMO shortly after randomization (mean=4.3 min). This would not have been possible in a real clinical setting. We designed the experiment in this way in order to gain the clearest possible insight into the potential effects of ECMO. In future studies a time delay could be inserted to simulate the duration of cannulation or cannulation could actually be performed once cardiac failure sets in. On the other hand our studies also revealed that ECMO could be used, even after a period of cardiac arrest, as the time spent on ventilator in the ventilator group delays the initiation of ECMO treatment.

We decided that it would involve too high a risk for the medical staff and the crew onboard the Hercules aircraft, to perform the poisoning of the pig onboard. No immediate escape or access to non-toxic air would be possible in case of a major leak of CO into the cabin of the aircraft. Therefore, we decided to poison the pig in a hangar close to the runway. This, however, had the side effect, that we could not perform the experiment in the same manner as in study 2, where CO intoxication was increased gradually until cardiac failure. Instead, HbCO was kept at around 50% to ensure severe poisoning but not initiating cardiac failure, as this would compromise our attempt to perform the cannulation while airborne. In a clinical

setting, the drop in HbCO would also have been present during the transportation from a medical facility to Airfield.

Due to the nature of this experiment, where multiple cooperating teams and a military aircraft were used, only one flight with the described setup of study 3 was possible during this PhD project. It would have improved credibility of the data, if multiple flights could have been performed, and further insight into the potential challenges of in-air cannulation might have been gained. Planning and execution of test flights are a demanding and costly task. A different solution would have been, using a hypobaric chamber for the tests. Though, as this is a feasibility study, several factors with influence on feasibility would have been hard to recreate in a hypobaric chamber; turbulence, vibration and G-forces.

7 CONCLUSIONS

The work of this thesis resulted in improved knowledge regarding the epidemiology of CO poisoning in Denmark. A new treatment option in which ECMO was used was investigated using a porcine model. This treatment seemed promising, although further research is needed. In addition, a feasibility study was performed where the treatment methodology from Study 2 was implemented successfully during a flight with a fixed wing aircraft.

The three studies included in this PhD thesis, lead to several important conclusions.

In study 1, it was demonstrated that around 1,000 patients suffer from CO poisoning/smoke poisoning each year, and around 100 people die from this in Denmark. Treatment with HBO therapy was not associated with increased or decreased survival when taking co-morbidities, sex and age into account. The co-morbidities that are associated with increased mortality, or the risk of being poisoned in the first place, are generally factors that affect cognitive or physical function i.e. age, alcohol abuse, psychiatric disease, arterial embolisms and cerebrovascular disease. Approximately 50% of those who died, committed suicide and in these cases, the preferred source of CO-exposure was from gas form and not from fire smoke.

Study 2 demonstrated, in a porcine experimental model, superior survival using ECMO vs. normobaric oxygenation with 100% oxygen for CO poisoning with subsequent cardiogenic shock. It was also demonstrated that even in case of cardiac arrest, resuscitation efforts followed by ECMO treatment may secure survival, and that it was possible to achieve a significantly higher PaO_2 using ECMO vs. ventilator with FiO_2 at 100%. Cardiac failure was not a result of increased PVR.

In study 3, it was demonstrated that it is feasible to transfer the findings from study 2 to an aeromedical setting. Use of ECMO on a fixed wing aircraft to treat CO poisoning in a porcine model was possible. Cannulation and ECMO treatment was successfully performed, during cardiac arrest, while airborne.

8 FUTURE PERSPECTIVES

Altogether, the studies included in this PhD thesis have generated several questions for further research.

Although a decreasing trend in the number of fatalities from CO poisoning has been observed each year, it is still a relatively frequent cause of hospitalization and leads to subsequent mortality and morbidity. The relationships between the co-morbidities/CO poisoning and death established in this study provide information about which patient categories are at an increased risk of being poisoned and have the highest mortality. Using this information, preventive efforts may be established, such as the installation of smoke/CO detectors, and close follow up following discharge should be considered. In Study 1, no survival benefits of HBO treatment were observed. Further studies examining this topic are required to clarify the effects of HBO. Since a substantial selection bias is hard to avoid because HBO treatment is only available at one site in Denmark. Future studies should employ a randomized controlled trial design and include patients throughout Denmark. The addition of clinical data would improve the possibilities to draw a solid conclusion.

The future use of ECMO for CO poisoned patients with cardiac failure seems promising, although additional research is required. As several limitations in transferring knowledge obtained from animal studies to the treatment of human patients exist, a study protocol that involves human victims of CO poisoning must be designed. However, additional animal studies may be needed prior to conducting human trials. A study assessing neurological outcomes would be beneficial to avoid resuscitating patients to a poor quality of life, which is associated with severe ischemic brain injury. Emerging biomarkers for cerebral injury, such as neuron-specific enolase or S100b, might be beneficial for these experiments (167). Additionally, several possibilities exist to optimize the ECMO protocol before testing it in a hospital setting. When the patient is connected to extracorporeal circulation, the blood can be treated to facilitate a faster reduction in HbCO levels. Would illumination with a strong light source with wavelength between 532-628 nm be an option (168)? These wavelengths have poor penetration in tissue, but extracorporeal use would bypass this limitation. Would the addition of CO_2 to the oxygenator be beneficial (169)? Would simultaneous treatment with antioxidants be effective? Could ECMO and HBO be administered at the same time? All these questions will inspire several additional research projects.

The possibilities of using ECMO during long-distance transport in fixed wing aircrafts for CO poisoned victims may facilitate the safer transport of these patients from the area of intoxication to a capable health care facility that may offer HBO, such as in a military setting in which smoke/CO poisoned victims are transported from an area

of conflict or a situation with a massive number of casualties, i.e., a nightclub fire (154). The potential to perform cannulation while airborne might allow the transport of critical patients who otherwise would have been ruled too unstable to move because ECMO can be used as a backup strategy. The use of ECMO treatment as a rescue therapy might also be beneficial when transporting patients with other injuries that impair cardiopulmonary functions, i.e., blast-injured lungs, ARDS, pulmonary embolism and hypothermia. In a military setting, the implementation of ECMO may shorten the evacuation chain by one or two steps, and thus forward medevac and tactical medevac would be combined into one flight to a Role 4/5 health care facility. During this flight, en route damage control surgery may be performed with the possibility of administering ECMO as a treatment option.

Clearly, more research in this area is required and, although some disadvantages exist, we suggest the use of a hypobaric chamber to simulate pressure conditions at altitude and make further studies more feasible.

Altogether, this PhD thesis reveals the substantial potential for further research in the field of CO poisoning, with promising signs that additional research may lead to the development of improved treatments in the future.

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10 APPENDICES: PUBLISHED PAPERS AND MANUSCRIPTS IN REVIEW

Appendix A.

Study 1:
Simonsen C, Thorsteinsson K, Mortensen RN, Torp-Pedersen C, Kjærgaard B, Andreasen JJ. Carbon monoxide poisoning in Denmark with focus on mortality and factors contributing to mortality. Submitted to PLOS One.

Appendix B.

Study 2
Simonsen C, Magnusdottir SO, Andreasen JJ, Rohde MC, Kjærgaard B. ECMO improves survival following cardiogenic shock due to carbon monoxide poisoning - an experimental porcine model. Scand J Trauma Resusc Emerg Med 2018;26:103. doi:10.1186/s13049-018-0570-6.

Appendix C.

Study 3
Simonsen C, Magnusdottir SO, Andreasen JJ, Bleeg RC, Lie C, Kjærgaard B. Long distance transportation of CO-poisoned patients on ECMO seems possible – a porcine feasibility study. Submitted to Air Medical Journal.

Appendix A. Study 1

Simonsen C, Thorsteinsson K, Mortensen RN, Torp-Pedersen C, Kjærgaard B, Andreasen JJ. Carbon monoxide poisoning in Denmark with focus on mortality and factors contributing to mortality.

Submitted to PLOS One.

Appendix B. Study 2

Simonsen *et al. Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine* (2018) 26:103
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Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine

ORIGINAL RESEARCH Open Access

ECMO improves survival following cardiogenic shock due to carbon monoxide poisoning - an experimental porcine model

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Abstract

Background: Severe intoxication with carbon monoxide (CO) is extremely lethal and causes numerous deaths due to cardiac or respiratory failure. Conventional intensive treatment may not be sufficient. The aim of this study was to investigate the treatment effect of extracorporeal veno-arterial extracorporeal membrane oxygenation (ECMO) following severe CO poisoning in an experimental porcine model.

Methods: A total of twelve pigs were anaesthetized, routinely monitored and intoxicated by inhalation of CO until the beginning of cardiac failure and randomized to a treatment (ventilator using an FiO_2 of 100% or ECMO). In the case of cardiac arrest, advanced resuscitation using standard guidelines was performed for at least 10 min. ECMO was also initiated in the ventilation group if the return of spontaneous circulation did not occur within 10 min. Lung tissue biopsies were obtained before and after CO intoxication.

Results: All animals in the ECMO group survived; however, one had to be resuscitated due to cardiac arrest. A single animal survived in the ventilator group, but five animals suffered from cardiac arrest at an average of 11.8 min after initiation of treatment. Conventional resuscitation failed in these animals, but four animals were successfully resuscitated after the establishment of ECMO.
A significant decrease was noticed in PO_2 with increasing HbCO, but there was no increase in pulmonary vascular resistance. No differences in H&E-stained lung tissue biopsies were observed.

Conclusions: The use of ECMO following severe CO poisoning greatly improved survival compared with conventional resuscitation in an experimental porcine model. This study forms the basis for further research among patients.

Keywords: Carbon monoxide poisoning, Smoke poisoning, Extracorporeal membrane oxygenation, Hyperbaric oxygenation, Cardiac failure, Respiratory failure, Pulmonary vascular resistance

Introduction

Carbon monoxide (CO) is extremely treacherous; invisible and without smell or taste, this lethal gas overtakes people without warning. Negligible CO concentrations occur in the atmosphere, but large amounts of CO form during the insufficient combustion of organic material, and a primary risk of exposure is the inhalation of smoke from fires [1]. Other significant sources of CO poisoning include residential heat sources, suicide/attempts and occupational exposure [2–5].

Approximately 50,000 annual contacts with emergency departments in the US are due to CO poisoning, resulting in approximately 2700 deaths [6, 7]. Surviving patients may suffer from increased risk of developing neurological symptoms, e.g., extrapyramidal symptoms, encephalopathy and cardiac insufficiency [8–10].

The rationale behind all currently established treatments for CO poisoning is to elevate the partial pressure of oxygen in the blood, favouring the formation of HbO_2 instead of HbCO, and to increase the oxygen content in the blood [11, 12]. In mild cases, this is achieved by

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administering supplementary normobaric oxygen (NBO) for inhalation. In more severe cases, hyperbaric oxygen treatment (HBO) is used [11]. HBO can further increase blood oxygen content, resulting in a decreased half-life of HbCO, but the protocol for when to offer HBO differs greatly internationally [13].

CO poisoning may have a profound effect on both respiratory and cardiac function, but although HBO increases blood oxygen tension and decreases HbCO half-life, the benefits of HBO remain highly debated [14–17]. Conventional intensive treatment methods may not be sufficient, and the aim of the present study was to investigate the treatment effect of extracorporeal veno-arterial extracorporeal membrane oxygenation (ECMO) following severe CO poisoning in an experimental porcine model. The porcine model was chosen due to the close resemblance between porcine and human anatomy/physiology [18]. We hypothesized that (ECMO) would improve survival.

Methods

Ethical statement

This study was carried out in accordance with Danish and European legislation regarding the use of animals for research purposes. The experiments were approved by the Danish Animal Experiments Inspectorate (J.nr. 2016-15-0201-01064). At all times, a veterinarian was present, and all participants had training in laboratory animal science prior to experimentation.

Experimental animals and instrumentation

All experiments were carried out at the Biomedical Research Laboratory at Aalborg University Hospital, Denmark. The research animals were 12 female pigs (Danish Landrace) with an average weight of 48 kg (range 45–51 kg) and approximately 90 days old. The animals were housed in nearby boxes at the laboratory for acclimatization up to 7 days prior to the experiment. During this period, the animals had access to food/water and were attended to by laboratory staff several times each day. Premedication with Zoletil, an anaesthetic combination drug containing equal concentrations of Tiletamine and Zolazepam, was used. Anaesthesia, which was similar in both study groups, was maintained with continuous intravenous infusion of fentanyl and propofol based on weight, with minor adjustments to ensure that anaesthesia was sufficient. The animals were intubated using a 6.5-mm cuffed endotracheal tube and connected to a ventilator (Dameca DREAM, Rødovre, Denmark). Tidal volume was calculated using 8 mL/kg and the respiratory rate (RR) was adjusted according to blood CO_2 levels (14–17/min) and reduced whenever ECMO was running. FIO_2 was set at the lowest level possible while still achieving blood PO_2 levels within a normal range. To avoid atelectasis, positive end-expiratory pressure (PEEP) was fixed at five cm H_2O and recruitment was performed regularly by increasing PEEP to 10–15 cm H_2O. A small venous catheter was placed in one ear vein to facilitate the infusion of fentanyl and propofol as primary anaesthesia during the remainder of the experiment. Throughout the experiment, fluid was administered according to existing guidelines for porcine anaesthesia [19, 20]. A bladder catheter with a thermal sensor was inserted and used for monitoring diuresis and core temperature. To detect any possible arrhythmias, constant electrocardiography was performed. Real-time arterial pressure measurements were achieved using an arterial catheter connected to a pressure transducer inserted into the right carotid artery. The same catheter was used for drawing blood for analysis.

After a full sternotomy, arterial catheters were inserted into the pulmonary artery and the left atrium to measure pressure differences over the pulmonary vascular system. The catheters were also used for drawing blood for analysis. Using an articulating dissection instrument (Wolf Lumitip Dissector™, AtriCure, Mason, Ohio, US)-modified for multiple use as a guide, a division of the fibrous tissue connecting the aorta and the pulmonary artery was made, allowing for the placement of a sonography probe (16–18 mm, MediStim, Copenhagen, Denmark) around the main pulmonary artery. The probe was connected to a flow monitor (MediStim, Copenhagen, Denmark), enabling real-time measurements of the cardiac output.

The flow through the main pulmonary artery was used as cardiac output and for calculating the pulmonary vascular resistance (PVR). PVR was calculated using the following formula: PVR = (80 x (Mean Pulmonary Arterial Pressure- Left Atrial Pressure))/Pulmonary Blood Flow. The right femoral artery and vein were exposed after surgical incision, and after heparin injection (30,000 IE), a 15 French cannula (Medtronic, Minneapolis, Minnesota, US) was inserted into the artery for infusion of blood from the extracorporeal system. Drainage to the system was achieved using a 21 French cannula (Medtronic, Minneapolis, Minnesota, US) inserted over a guide wire into the right jugular vein.

For extracorporeal circulation, we used a prototype centrifugal pump to drive the extracorporeal circulation through an oxygenator (QUADROX adult, Maquet, Rastatt, Germany). Oxygen flow to the oxygenator was set at a constant level of 2 L/min with 100% oxygen. Extracorporeal blood flow was measured using an ultrasonic flowmeter (Sono TT, em-tec GmbH, Finning, Germany). The pump was initially set to 3000 rounds per minute (RPM), resulting in a mean flow of 2.4 L/min. By using two additional Y-connectors, a shunt in the external circulation was created, allowing us to initiate/stop external circulation quickly without tampering with pump settings.

CO was delivered from a pressure cylinder with an attached pressure reduction valve. Through "air" tubes, CO gas was connected to 1) a CO monitor (Exhaust Emission Gas Analyser, Model SV-5Q, China Coal, Shaanxi, China) and 2) the research animal via the ventilator, forming a closed system to avoid leakage of CO into the operating theatre. When administering CO, the valve was opened briefly with intervals to avoid overdosing and to keep the inhalation concentration at a level of approximately 1–2%. CO administration was stopped permanently at the time of randomization. Conventional arterial blood gas analyses were made regularly using a blood gas analyser (ABL800 FLEX Series, Radiometer Medical, Brønshøj, Denmark), allowing us to track changes and to keep track of HbCO during the experiment. Constant CO monitoring with an alarm was used to secure the safety of laboratory personnel in the room.

Experimental protocol

Animals were randomly assigned to the study groups following simple randomization procedures (computerized random numbers) by a third party. Allocation concealment was kept blinded for the study personnel who were going to implement assignments at the time that cardiac failure was evident (defined as cardiac output decreased to 50%), which was taken as a surrogate measure of severe CO intoxication. At this point, a sequential numbered, sealed, opaque envelope was opened. Blinding to the allocated arm was not possible due to the nature of the experiment. The primary outcome in the model was survival. The histological effect on lung tissue and changes in PVR were used as secondary outcomes.

Cardiac arrest, defined as systolic blood pressure below 25 mmHg, was treated using advanced resuscitation according to the 2015 guidelines of the European Resuscitation Council [21, 22]. However, chest compressions were replaced by internal cardiac compressions, and direct current defibrillation was attempted using internal paddles (Zoll Pro Pac MD, ZOLL Medical Corporation, Chelmsford, Massachusetts, US). If resuscitation failed in the ventilator group (defined as no return of spontaneous circulation (ROSC) and a lack of any signs of improvement in the condition within 10 min), ECMO was established. Weaning from ECMO in both groups was not attempted until HbCO was less than 10%. Weaning was not considered successful unless 10 min of off-pump circulation was completed without cardiac or respiratory failure. All animals were euthanized using pentobarbital intravenous injection after completion of the experiments.

Tissue samples of approximately eight cm^3 were taken from the lungs close to the pleura at different sites prior to CO poisoning. Similar samples were taken after intoxication at the time of randomization. These samples were preserved using formalin and analysed microscopically at the Department of Forensic Medicine, Aarhus University, after slicing and staining with haematoxylin and eosin (H&E).

Statistical analysis

We used the "resource equation" method for sample size calculation in the present study, as it was not possible to assume anything about the effect size or to determine standard deviations from previous studies [23]. According to this method, the value "E" was measured by the following formula: E = Total number of animals − Total number of groups. Any sample size that maintained "E" between 10 and 20 was considered adequate. To avoid unnecessary wastage of resources and comply with ethical issues, we kept the number of animals included in this pilot study to six in each group, i.e., "E": 12−2 = 10.

For statistical analysis, we used the open source freeware program R, version 3.4.3/R-studio and IBM SPSS, version 25. Group comparisons at baseline and at the point of randomization were made using an unpaired t-test. Tests for normality were performed by visual inspection of qq-plots of all variables and Levene's test for equality of variances. A paired-samples t-test was conducted to compare mean pO_2 at baseline and mean pO_2 at the point of randomization. A simple linear regression was constructed to predict pO_2 based on HbCO. Similarly, we constructed a linear regression to predict pulmonary vascular resistance (PVR) based on HbCO and PVR based on pO_2. An exponential regression was performed to describe the correlation between lactate and HbCO.

Results

There were no significant differences between the study groups at baseline (Table 1) and at the time of randomization (Table 2). The mean time of the duration of CO intoxication was 53 min: 51.0 min for the ECMO group (SD = 13.3) and 56.5 min for the ventilator group (SD = 14.8), $p = 0.97$.

All animals survived in the ECMO group for at least 10 min after weaning from ECMO once HbCO was below 10%, although one had to be resuscitated due to a cardiac arrest that occurred immediately after the initiation of external circulation (ROSC after 17 min). The mean time from the identification of heart failure to the initiation of ECMO treatment was 4.3 min. Only one animal survived in the ventilator group, and five suffered from cardiac arrest at an average of 11.8 min after the initiation of treatment. It was not possible to resuscitate any of these animals by conventional means within 10 min of cardiac arrest. However, after initial resuscitation attempts were abandoned, we established ECMO treatment and successfully managed to resuscitate four of these animals (Fig. 1). No adverse events occurred. Time

Table 1 Baseline characteristics

	Ventilator (*n* = 6)		ECMO (n = 6)		Difference	
		CI (95%)		CI (95%)		*P*-value
Weight (kg)	48.7	46.2–51.1	47.5	44.5–50.5	1.17	0.46
HbCO (%)	2.9	2.3–3.5	3.2	2.5–3.8	−2.5	0.47
pH	7.41	7.33–7.49	7.42	7.36–7.47	−0.01	0.86
Hb (mmol/L)	4.58	4.08–5.09	4.87	3.84–5.89	−0.28	0.54
pCO_2 (kPa)	5.37	4.85–5.88	5.27	4.70–5.84	0.10	0.75
pO_2 (kPa)	11.53	8.33–14.74	10.68	9.58–11.78	0.85	0.53
Lactate (mmol/L)	1.23	0.72–1.75	1.1	0.68–1.53	0.13	0.62
Temperature (°C)	37.3	35.8–38.9	37	36.6–37.5	0.28	0.67
Cardiac output (L/min)	3.45	2.77–4.13	3.72	2.84–4.60	−0.27	0.55
MAP (mmHg)	78.5	62.9–94.1	85.7	76.1–95.3	−7.2	0.34
HR (beats/min)	71.2	47.5–94.8	73.5	57.2–89.8	−2.3	0.84
MPAP (mmHg)	24.5	23.1–26.0	25.8	20.0–31.6	−1.3	0.58
MLAP (mmHg)	10.2	8.9–11.4	10.5	9.4–11.6	−0.3	0.61
PVR (dyn·s·cm^{-5})	344.2	238.7–449.6	325.2	248.8–401.5	19	0.72

The table shows baseline characteristics of essential values in the ventilator group vs the ECMO group. *MAP* Mean Arterial Pressure, *HR* Heart Rate, *MPAP* Mean pulmonary Pressure, *MLAP* Mean left atrial pressure, *PVR* Pulmonary Vascular resistance

on ECMO was 182.5 min (SD = 21.9) for the ECMO group and 201.6 min (SD = 63.5) for those in the ventilator group that ended up on ECMO after failure of conventional resuscitation, $p = 0.96$. The mean time for HbCO to fall below 0.1 after intoxication was 123.7 min (SD = 20.0) for the ECMO group and 163.7 min (SD = 15.2) for the ventilator group, $p = 0.56$.

Lactate concentrations in the blood increased exponentially as HbCO increased, $p < 0.001$ (R^2 of 0.562) (Fig. 2a). A significant regression equation was found when comparing pO_2 with HbCO, $p < 0.001$, (R^2 of 0.496). The predicted pO_2 was equal to 109.2 mmHg minus 10.4 mmHg for every 0.1% increase in HbCO (Fig. 2b). There was a significant difference in pO_2, mean 83.1 mmHg (95% CI: 72.8–93.0) at baseline vs. mean 26.3 mmHg (95% CI: 20.3–26.3) at randomization, $p < 0.001$. We did not find a significant linear regression equation, $p = 0.54$ ($R^2 = 0.008$) when exploring the association between PVR and HbCO (Fig. 2c). However, mean PVR was the lowest at HbCO = 54% corresponding to a mean PVR of 200

Table 2 Characteristics at point of randomization

	Ventilator (*n* = 6)		ECMO (n = 6)		Difference	
		CI (95%)		CI (95%)		*P*-value
HbCO (%)	69.7	59.4–80.0	67.9	49.6–86.2	−2.0	0.83
pH	7.26	7.16–7.36	7.31	7.23–7.39	−0.05	0.34
Hb (mmol/L)	5.85	4.98–6.72	6.17	5.21–7.12	−0.32	0.54
pCO_2 (mmHg)	37.7	29.0–46.4	39.2	34.4–44.0	−1.4	0.72
pO_2 (mmHg)	34.5	21.5–47.6	24.4	17.4–31.4	10.1	0.12
Lactate (mmol/L)	7.33	6.45–8.22	6.27	5.27–7.26	1.07	0.07
Temperature (°C)	37.4	36.1–38.8	37.1	36.4–37.7	0.35	0.56
Cardiac output (L/min)	1.57	1.17–1.96	1.34	0.43–2.25	0.23	0.57
% of base line	49.5	37.5–61.4	41.33	6.39–76.26	8.13	0.59
MAP (mmHg)	46.3	35.1–57.6	38.8	32.3–45.4	7.5	0.18
HR (beats/min)	102.5	74.8–130.2	108.17	84.4–131.9	−5.67	0.70
MPAP (mmHg)	20,0	16.6–23.5	16.2	12.5–19.8	3.83	0.08
MLAP (mmHg)	8.2	5.6–10.8	7.8	5.4–10.3	0.33	0.82
PVR (dyn·s·cm^{-5})	657.3	316.2–998.5	579.8	207.8–951.9	77.5	0.70

The table shows characteristics of essential values at point of randomization in the ventilator group vs the ECMO group. *MAP* Mean Arterial Pressure, *HR* Heart Rate, *MPAP* Mean pulmonary Pressure, *MLAP* Mean left atrial pressure, *PVR* Pulmonary Vascular resistance

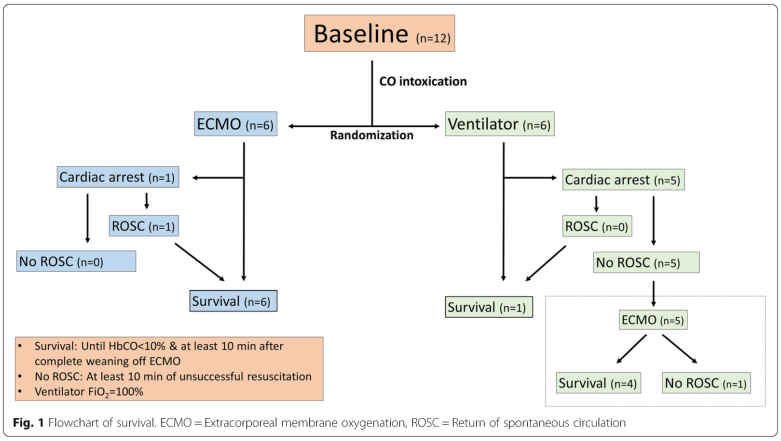

Fig. 1 Flowchart of survival. ECMO = Extracorporeal membrane oxygenation, ROSC = Return of spontaneous circulation

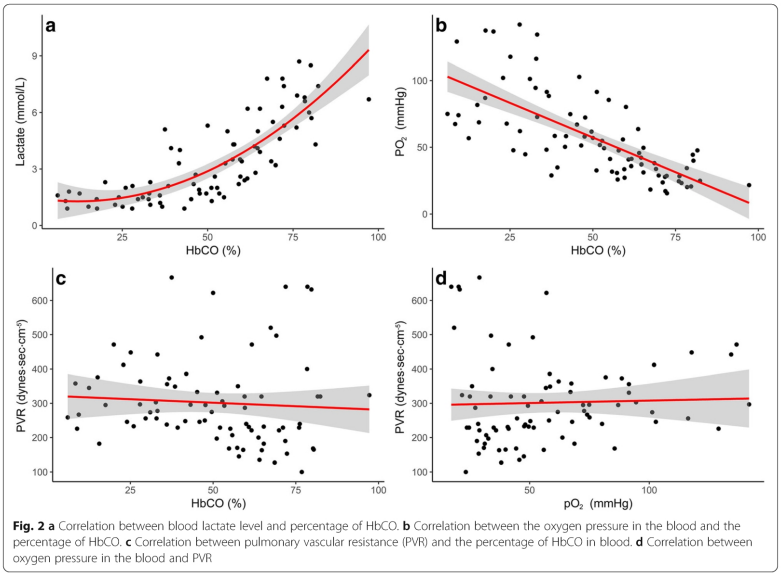

Fig. 2 **a** Correlation between blood lactate level and percentage of HbCO. **b** Correlation between the oxygen pressure in the blood and the percentage of HbCO. **c** Correlation between pulmonary vascular resistance (PVR) and the percentage of HbCO in blood. **d** Correlation between oxygen pressure in the blood and PVR

dyn·s/cm^5 (95% CI: 148–252) compared to the baseline mean PVR of 319 dyn·s/cm^5 (95% CI: 261–378) (Fig. 3a). This difference was statistically significant ($p < 0.001$). No correlation was found between pO_2 and PVR, $p = 0.76$ ($R^2 = -0.001$) (Fig. 2d). Regarding the highest achieved pO_2 dependent on treatment, the highest pO_2 on ECMO was 551.3 mmHg (95% CI: 487.5–615.0) versus the highest pO_2 on a ventilator at 99.8 mmHg (95% CI: 0–283.5) (Fig. 3b). In surviving animals, we were able to increase pO_2 from a mean of 27.0 mmHg (95% CI: 19.5–30.8) at the time of randomization to a mean of 209.3 mmHg (95% CI: 102.8–315.0), $p = 0.003$.

There were no microscopic differences in H&E-stained lung tissue biopsies obtained prior to CO intoxication versus after (Fig. 4). No intra-alveolar fluid accumulation and no signs of inflammation were evident.

Discussion

In this study, we showed that ECMO treatment in severe cases of CO poisoning greatly improved survival compared with conventional resuscitation in an experimental porcine model. Thus, ECMO may serve as a treatment option in addition to conventional treatment following severe CO poisoning. The use of HBO is only possible in a limited number of hospitals within each country, and treatment can only be offered to a fraction of the population without the need for interhospital transportation. In contrast, ECMO treatment is available in mobile systems and can be transferred to the patients [24]. In Denmark, a highly mobile ECMO team exists, using helicopter assets from the Royal Danish Airforce when needed to reduce transport time.

The increased probability of survival, even following cardiac arrest and resuscitation, underlines ECMO treatment's ability to stabilize respiratory and cardiac function while proper restitution occurs. For practical reasons, we weaned the animals from ECMO as soon as HbCO was below 0.1%. In real clinical settings, more time would probably be advisable to allow for more complete restitution. We chose 10 min post-weaning as marker for survival because in our experience, subsequent circulatory failure would probably reoccur during this timeframe.

A large proportion of CO-poisoned patients may suffer from lung injuries from other components in smoke (e.g., nitrogen oxide gasses, hydrogen chloride) and thermal injuries from the inhalation of hot gases. In these cases, the benefits of ECMO would potentially be even greater, as current treatment with a ventilator and/or HBO rely on the lung diffusion capacity to ensure sufficient oxygen tension in the blood.

In a study of 18 patients who suffered from cardiac arrest due to CO poisoning, none of the patients who were subjected to HBO treatment after resuscitation survived hospitalization [14]. The authors concluded that "the prognosis of this condition should be considered when making triage and treatment decisions for patients poisoned to this severity", implying that the termination of treatment should be considered if cardiac arrest occurs in this patient category. The cause of this negative outcome may be explained by pulmonary insufficiency due to inhalation injuries from smoke/heat that make efficient gas exchange impossible, and a case report from 2017 indicated that patients with pulmonary insufficiency might experience longer HbCO half-life, diminishing the possible positive benefits of HBO treatment [25]. The results of the present study imply that survival may be possible if ECMO can be established.

The benefits of ECMO may be explained by the release of strain on the heart, lowering oxygen consumption and allowing sufficient restitution following ischaemia. Another favourable effect of using ECMO is

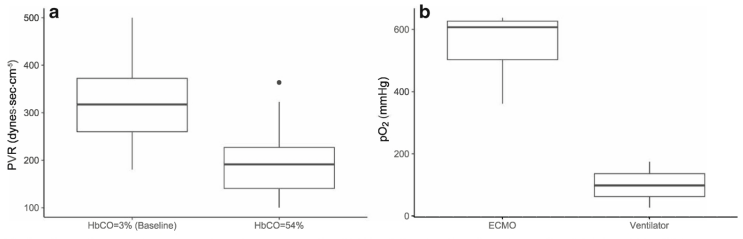

Fig. 3 **a** Comparison of pulmonary vascular resistance at baseline (HbCO at 3%) and at the point of heart failure (HbCO at 54%). **b** Highest achieved oxygen pressure dependent on the treatment group

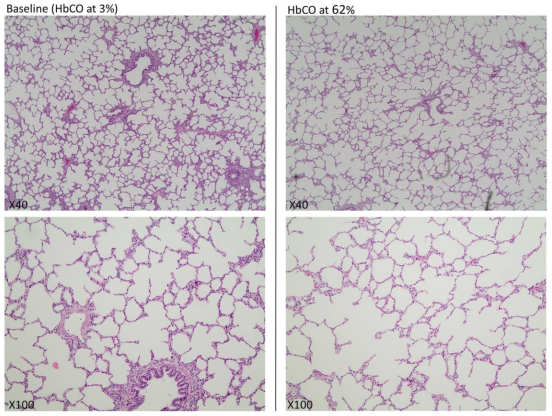

Fig. 4 Representative haematoxylin and eosin-stained slices of lung tissue at baseline and at a high level of CO intoxication

its non-dependence on the condition of the lungs and airways. ECMO has the potential to increase blood oxygen tension, diminishing ischaemia and favouring increased formation of oxyhaemoglobin and elimination of CO.

Prior to the experiments, we expected that PVR would increase during CO poisoning, contributing to cardiac insufficiency through a backward failure mechanism. However, this was not the case as the trend was towards a lower PVR during CO poisoning. It is possible that this can be explained by hypoxia induction of the relaxation of smooth muscle cells in resistance vessels in the pulmonary system, but separate experiments must be undertaken to clarify this. To the best of our knowledge, no previous studies regarding changes in PVR due to CO poisoning have been published.

We found a negative linear correlation between HbCO and O_2. Our initial presumption was that this might be due to a negative impact on the lung tissue, especially the diffusion barrier, making O_2 absorption progressively harder. This was not supported by the histological findings on the lung biopsies obtained prior to CO poisoning and compared individually with biopsies obtained after CO poisoning; no consistent differences were detected. The answer may be found on a molecular level, undetectable by the analysis of this experiment. Another hypothesis may be that CO causes shunting in the lungs, which is supported by our finding of decreased PVR.

Promising experiments have been made using light to decrease HbCO's half-life, and it would be simple to expose the oxygenator in the ECMO system to a strong source of light [26]. Other experiments have used O_3 instead of O_2 as oxygen supply to the oxygenator in the external circulation to decrease HbCO's half-life [27]. Some patients suffering from CO poisoning due to inhaling smoke will also suffer from cyanide poisoning [28]. A specific antidote for cyanide may be administered while ECMO stabilizes the patient, the effects of both CO and Cyanide diminish and the patient recovers.

A limitation of this study is that all animals were sacrificed at the end of the experiment due to ethical reasons. Thus, we had no ability to evaluate any neurological outcomes. Additionally, long-term mortality and morbidity could not be evaluated. In two case reports regarding successful ECMO support of patients suffering from severe CO poisoning with insufficient response to traditional ventilator therapy, no neurological deficits were detected during follow-up [29, 30]. There may be a theoretical risk of bias if efforts for resuscitation differed between study groups. However, we have no reason to believe this was the case as we strictly followed published resuscitation algorithms in both groups.

Precautions must be taken when inferring results from animal studies to human clinical settings; nevertheless, since this study involved large animals, we speculate that similar results may be obtained when humans are treated. Furthermore, the benefits of using ECMO must be weighed against the risk of potential complications.

Conclusion

The use of VA-ECMO following severe cases of CO poisoning with cardiogenic shock greatly improved short-term survival compared with conventional resuscitation in an experimental porcine model. This study forms the basis for further research among patients.

Abbreviations

CO: Carbon monoxide; ECMO: Extracorporeal membrane oxygenation; FiO_2: Fraction of Inspired oxygen; H&E staining: Haematoxylin and eosin staining; HBO: Hyperbaric oxygen; NBO: Normobaric oxygen; PEEP: Positive end-expiratory pressure; PVR: Pulmonary vascular resistance; ROSC: Return of spontaneous circulation; RR: Respiratory rate

Acknowledgements

Not applicable

Ethical approval and consent to participate

This study was carried out in accordance with Danish and European legislation regarding the use of animals for research purposes. The experiments were approved by the Danish Animal Experiments Inspectorate (J.nr. 2016-15-0201-01064). At all times, a veterinarian was present, and all participants had training in laboratory animal science prior to the experiments.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CS Literature search, data collection, study design, analysis of data, manuscript preparation, review of manuscript. SOM Literature search, data collection, study design, manuscript preparation, analysis of data, review of manuscript. JJA Literature search, data collection, study design, analysis of data, manuscript preparation, review of manuscript. MCR Analysis of data (especially microscopic evaluation of lung biopsies), manuscript preparation, review of manuscript. BK Literature search, data collection, study design, analysis of data, manuscript preparation, review of manuscript. All authors read and approved the final manuscript.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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Appendix C. Study 3

Simonsen C, Magnusdottir SO, Andreasen JJ, Bleeg RC, Lie C, Kjærgaard B. Long distance transportation of CO-poisoned patients on ECMO seems possible – a porcine feasibility study.

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